*Supplement to the*
*"Proceedings of the Indian Academy of Sciences"*

# PROCEEDINGS

OF THE

# INDIAN ACADEMY OF SCIENCES

VOL. XL

SECTION B

BANGALORE CITY
PRINTED AT THE BANGALORE PRESS, MYSORE ROAD
1955

Q
73
.I36
Amer. B
v.40

# CONTENTS

## SECTION B—VOL. XL

PAGE

### No. 1—July 1954

**Bio-Assay of Heavy Metals by *Aspergillus niger*—Sensitivity of a New Strain** . . . . . (Miss) L. Saraswathi Devi 1

**Complete Enumeration Experiment in Groundnut Crop for Developing the Optimum Shape and Size of Samples for Crop Weather Observations** . . . . . A. K. Mallik, V. K. Subramanian and R. Venkatraman 8

**Additions to Fungi of Madras—XVI** . . . . . T. S. Ramakrishnan and N. V. Sundaram 17

### No. 2—August 1954

**Growth Studies in *Saccharum officinarum*. VII. Number of Stalks in the Clump in Relation to Weight of Plant, Length of Millable Stalk and Quality Ratio of the Crop** . . . P. C. Raheja and G. S. Sekhon 25

**Studies on Indian Ichneumonidæ (Hymenoptera). Sub-family: Banchinæ. Part I. Tribe Glyptini** . . . E. S. Narayanan and Kundan Lal 38

**The Effect of Prawn Protein on the Bone of Mature Rats** . . . . . T. C. Appanna and S. C. Devadatta 44

**Preliminary Studies on Certain Changes in the Plankton and Hydrological Conditions Associated with the Swarming of *Noctiluca*** . . . . . R. Raghu Prasad and R. Jayaraman 49

**The Problem of the Apparent Discontinuous Distribution of *Harpodon nehereus* (Hamilton)** . . . . . B. Sundara Raj 58

### No. 3—September 1954

**Studies on the Life-History of *Trichuris ovis* (Abildgaard, 1795) (Fam. Trichuridæ: Nematoda)** . . . . . Gobind Singh Thapar and Kr. Suresh Singh 69

### No. 4—October 1954

**The Post-Larval Development of the Shore Crab *Ocypoda platytarsis* M. Edwards and *Ocypoda cordimana* Desmarest** . . . . . Miss K. G. Raja Bai Naidu 89

**Pathological Studies of *Glæosporium psidii* Causing Die-back of Guavas** . . . . . R. N. Tandon and R. K. Agarwala 102

PAGE

**Desoxyribonucleic Acid (DNA) Content in the Female Gametophyte and Nucellus of *Rhæo discolor*** . . . . . M. Nagaraj 110

**Soil Amendments and Phosphate Availability** . . . . . G. P. Gokhale, M. M. Kibe and N. Narayana 116

**No. 5—November 1954**

**The Effect of Ions on the Contractile Mechanism of Unstriated Muscle and its Relation to Tonus** . . . Sunita Inderjit Singh and Inderjit Singh 125

**The Neurosecretory System of the Fruit Fly *Chætodacus cucurbitæ* Coq. I. Distribution and Description of the Neurosecretory Cells in the Adult Fly** . . . . . K. K. Nayar 138

**The Intimate Nature of Muscular Contraction** . . . . . Sunita Inderjit Singh and Inderjit Singh 145

**No. 6—December 1954**

**Bacteriology of Off-Shore Sea-Water of the West Coast** . . . . . R. Venkataraman and A. Sreenivasan 161

**Microchromatography. Part II. Detection of Trace Elements in Biological Media** . . . . . K. Lakshminarayanan 167

**Photoperiodism in Rice. III. Effect of Short Day-Length on Four Late-Winter Varieties of Rice of Orissa** . . . . . Gadadhar Misra 173

**Studies on Indian Ichneumonidæ (Hymenoptera)** . . . . . E. S. Narayanan and Kundan Lal 183

# BIO-ASSAY OF HEAVY METALS BY *ASPERGILLUS NIGER*—SENSITIVITY OF A NEW STRAIN

By (Miss) L. Saraswathi Devi
(*University Botany Laboratory, Madras*)

Received November 18, 1953
(Communicated by Professor T. S. Sadasivan, F.A.SC.)

The ability of some fungi to utilize minute traces of some heavy metals has been taken advantage of in bio-assay of these metals in soils, plant materials, chemical compounds, culture media, etc. (Mulder, 1939; Steinberg, 1945; Nicholas and Fielding, 1947, 1949, 1950, 1951; Nicholas, 1950; Hewitt and Hallas, 1951; Donald, Passey and Swaby, 1952 *a* and *b*). The sensitivity of these organisms has, however, varied; but in most cases where they have been used, the accuracy of the quantitative estimation of the elements has been greater than the results obtained by chemical analysis (Nicholas and Fielding, 1951; Hewitt and Hallas, 1951).

In this laboratory the purity of the culture medium to be used in trace element essentiality studies has been tested biologically, using the standard 'M' strain of *Aspergillus niger*. Simultaneously, a strain of the same fungus, isolated in this laboratory, was compared with the standard strain, as regards its sensitivity. The striking contrast shown by these two strains in their response to deficiencies of some heavy metals is presented.

## Materials and Methods

### *Strains used*

(i) *Aspergillus niger* van Tiegh. 'M' strain kindly supplied by Dr. D. J. D. Nicholas, Long Ashton Research Station, University of Bristol; (ii) *Aspergillus niger* van Tiegh. 'M.U.B.L. 1' (Madras University Botany Laboratory, 1), a strain isolated in this laboratory from the rhizosphere of *Cajanus cajan*, identified and kindly furinshed by Mr. V. Agnihothrudu. The cultures were maintained on 2% potato-sucrose-agar.

### *Glassware*

Only Pyrex glassware was used throughout. Culture flasks were 500 ml. conical, Erlenmeyer type and were covered by 100 ml. squat beakers inverted over the open necks. The plugging was done by packing an annular ring of cotton in the space between the beaker and the neck below the rim and

all glassware used were thoroughly cleaned and dried before each experiment (Hewitt and Hallas, 1951). Final rinsing was done in water distilled thrice over in Pyrex all-glass stills.

*Distilled water*

Thrice Pyrex glass distilled water was used in the preparation of media as well as micro-nutrient solutions.

*Basal medium*

A sucrose-nitrate medium (derived from high quality analytical reagents) of the following formula was used:

| | |
|---|---|
| $KNO_3$ .. .. | 10 g. |
| $KH_2PO_4$ .. .. | 5 g. |
| $MgSO_4.7H_2O$ .. | 2.5 g. |
| Sucrose .. .. | 50 g. |
| Distilled water .. | 1 litre |

*Purification of basal medium*

The $Al_2O_3$ (B.D.H. Chromatographic analysis material) method devised by Donald, Passey and Swaby (1952 *b*) was adopted to study deficiencies of Fe, Zn, Cu and Mn, and the $H_2S$ co-precipitation method advocated by Hewitt and Hallas (1951) for Mo deficiency studies, double purification having been carried out in the latter case. All filtrations were done using acid-washed No. 42 Whatman* filter-paper. The pH of the purified medium was adjusted to 5·0. 25 ml. aliquots were apportioned in the culture flasks, covered and sterilized at 15 lb. pressure for 15 minutes.

*Micronutrient solutions*

Six micro-nutrient stock solutions were prepared: a complete solution, with all the five heavy metals studied, (+ all) and five others in which Fe, Zn, Mn, Cu and Mo were omitted singly for the respective deficient cultures (— Fe, — Zn, — Mn, — Cu and — Mo series). The micro-nutrient solutions were added to each flask to give the following concentrations per 25 ml. of medium.

| | | |
|---|---|---|
| $FeCl_3.6H_2O$ .. .. | 75 $\mu$g. | Merck's guaranteed Reagent grade |
| $ZnSO_4.7H_2O$ .. .. | 50 $\mu$g. | |
| $CuSO_4.5H_2O$ .. .. | 15 $\mu$g. | |
| $MnSO_4.4H_2O$ .. .. | 7.5 $\mu$g. | |
| $(NH_4)_6Mo_7O_{24}.4H_2O$ .. | 10 $\mu$g. | |

The ' + all ' series served as the control, while a ' — all ' series was also run as a blank. A separate control (' + all ') was maintained for Mo studies.

*Inoculum*

Three loops of spores from a five-day old slant growth of each fungus, collected by a sterile platinum wire were dropped into 5 ml. of sterile treble-glass-distilled water and three drops of this spore suspension issuing from a 1 ml. sterile, Pyrex pipette, were used per flask.

## Experimental

The inoculated flasks were immediately incubated in the dark, at room temperature (27–29° C.) for six days, after which results were recorded.

*Results*

Both visual and dry weight assessments were made. Plate I shows the nature of growth and amount and colour intensity of sporulation; Table I gives the amount and percentage of growth of the two strains.

### Table I

*Showing the amount of and percentage growth of the two strains of* Aspergillus niger *van Tiegh. in complete and heavy metal deficient media*

Dry weight in mgm.

| Series | 'M' | | 'M.U.B.L. 1' | |
|---|---|---|---|---|
| | Dry weight | % growth | Dry weight | % growth |
| +all .. | 422·8 | 100 | 423·0 | 100 |
| —all .. | 22·7 | 5 | 41·4 | 9 |
| —Fe .. | 28·8 | 6·7 | 53·2 | 12 |
| —Zn .. | 30·5 | 7 | 52·2 | 12 |
| —Mn .. | 384·6 | 88·3 | 547·6 | 126 |
| —Cu .. | 172·6 | 39 | 200·6 | 46 |
| +all .. | 335·7 | 100 | 352·3 | 100 |
| —Mo .. | 196·5 | 58·6 | 370·5 | 105 |

## Discussion

The standard ' M ' strain of *Aspergillus niger* van Tiegh. has been used for bio-assay of heavy metals on account of its extreme sensitivity and stability with regard to its responses to heavy metal deficiencies. Growth and sporulation are the criteria considered. Marked decrease in growth as well as sporulation is characteristic of Fe-, Zn- and Mo-deficient cultures. Though Mn- and Cu-deficiencies do not decrease growth to the same extent as the other three, the amount and colour of spores are markedly affected in these cases (Nicholas and Fielding, 1951).

Judging from the nature and amount of growth and sporulation obtained in the present investigations with the ' M ' strain (*cf.* Plate I and Table I), good purification can be said to have been secured for both Fe- and Zn-deficiency studies. Growth in the respective deficient cultures was only 5 and 6% of that in the full culture. The degrees of purity obtained, with repeated trials (14 times) for Cu- and Mo-deficiency studies were only somewhat satisfactory; there was almost complete suppression of sporulation and the dry weights showed a 61 and 42% reduction in the respective series. The results obtained for Mn-deficiency studies, however, were not sufficiently encouraging.

Since the aim was only to secure a good degree of purity of medium for deficiency studies with Fe, Zn, Mn, Cu and Mo, no attempt at accurate quantitative estimations of these heavy metals in the purified medium was made. The percentages of growth, however, were sufficiently convincing.

Comparing the two strains of *Aspergillus niger* used in this study the responses to Fe- and Zn-deficiencies of both the strains were somewhat the same, although the ' M.U.B.L.' strain showed a slightly greater sensitivity than the ' M ' strain.

The most obvious contrast between the two strains was shown in the Mn-, Cu- and Mo-deficient cultures. The nature and amount of growth and sporulation shown by the standard strain in the ' —Mn ' culture, though not normal, showed the presence of a certain amount of this element in the purified medium. In spite of this being so, the M.U.B.L. strain showed very marked deficiency symptoms; sporulation was completely suppressed and the pure white mat was much wrinkled, giving the " cauliflower " appearance (Donald, Passey and Swaby, 1952 *b*). Hence, it seems evident that this strain requires a greater supply of Mn for normal metabolism, than the standard strain. In other words, the new strain is less sensitive to traces of manganese than the ' M ' strain. (The greater dry weight shown by the

'M.U.B.L.' strain in the Mn-deficient culture, in spite of marked visual deficiency symptoms, is a point of interest.)

The reverse was the case in the '— Cu' and '— Mo' series, the standard strain showing definite deficiency symptoms in both the deficient cultures. The mat was entirely white and cottony, and sporulation completely suppressed except for a ring of black spores at the edge of the mat, in the '— Cu' series, where the fungous growth was in contact with the culture flask. At the same time, the laboratory strain showed better growth and sporulation. This was very marked in the '— Mo' series where the dry weight was even greater than that of the control, which is again an interesting feature. The probabilities are that, either the new strain does not depend upon a supply of Mo for good growth and sporulation, or, it is able to utilize to its best advantage, the minute traces of this element which have been left over in the medium due to insufficient removal (as shown by the somewhat thick pellicle of the standard strain in '— Mo' culture), possibly indicating a greater sensitivity of this strain to traces of Mo.

In the '— Cu' series, both the strains showed deficiencies, indicated by dry weight as well as spore intensity. Sporulation was almost suppressed in the 'M' strain—a definite deficiency sign. Still, the laboratory strain showed a scant cover of yellowish brown spores, and a greater dry weight too, than the 'M' strain. It may, most probably, be that it is able to utilize Cu in even smaller amounts than the standard strain and it is, therefore, very likely that the new strain is more sensitive to small traces of copper.

The author is aware of the limitations under which the experiments were carried out and it is not held that the medium used was completely free of traces of the heavy metal nutrients under study, which ideal is difficult to attain in practice. Moreover, the response of the standard strain in the '— Mn', '— Cu' and '— Mo' series, though much encouraging, indicates minute traces of these three elements still left in the purified medium. A sufficient degree of purity was, however, obtained for Fe- and Zn-deficient studies. Further attempts aiming at a greater degree of purity for Mn, Cu and Mo studies are being made.

The experiment was repeated thrice and comparisons made only between results obtained in simultaneous trials. Though there were slight variations in the amount of growth, colour and intensity of spore cover in the different trials, the comparisons always led to the same inferences, *viz.*, 'M.U.B.L.' strain showed greater deficiency symptoms in the '— Mn' cultures whereas the 'M' strain exhibited greater deficiency signs in the '— Cu' and '— Mo'

series, though the difference in spore intensity between the two strains in the ' — Cu ' series tended to become somewhat less.

The dry weights of the laboratory strain in the ' — Mn ' and ' — Mo ' series were consistently higher than their respective controls. The behaviour of this new strain towards heavy metal deficiencies are being studied further.

## SUMMARY

The standard ' M ' strain of *Aspergillus niger* van Tiegh. which is being used in many laboratories as a biological test to detect the presence of some heavy metals in minute traces has been compared with a new strain of the same fungus, isolated in this laboratory, from the rhizosphere of *Cajanus cajan*, and designated as M.U.B.L. 1.

The new strain appeared to be less sensitive to traces of manganese, and more sensitive to copper, and perhaps molybdenum also, than the ' M ' strain. Further studies regarding the stability of the new strain and also attempts to secure a greater degree of purity of media are being conducted.

## ACKNOWLEDGEMENTS

The author is greatly indebted to Prof. T. S. Sadasivan and Dr. C. V. Subramanian for critical perusal of the manuscript, and to the University of Madras for the award of a studentship.

## REFERENCES

Donald, C., Passey, B. L. and Swaby, R. J. .. "Bio-assay of available trace elements from Australian soils," *Aust. J. agric. Res.*, 1952*a*, 3, 305–26.

——— .. "A comparison of methods for removing trace metals from microbiological media," *J. gen. Microbiol.*, 1952 *b*, 7, 211–20.

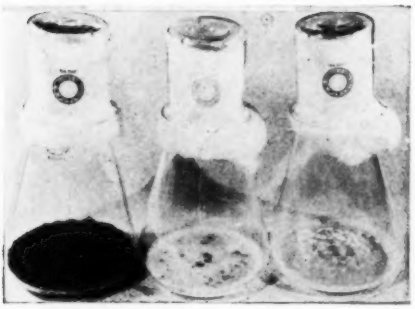
Hewitt, E. J. and Hallas, D. G. "The use of *Aspergillus niger* van Tiegh. M strain as a test organism in the study of molybdenum as a plant nutrient," *Plant and Soil*, 1951, 3, 366–408.

*Mulder, E. G. .. "On the use of micro-organisms in measuring the deficiency of copper, magnesium and molybdenum in soils," *Antonie van Leeuwenoek*, 1939, 6, 99–100.

Nicholas, D. J. D. .. "Use of *Aspergillus niger* for determining magnesium, copper, zinc and molybdenum in soils," *J. Sci. Food Agric.*, 1950, 1, 339–44.

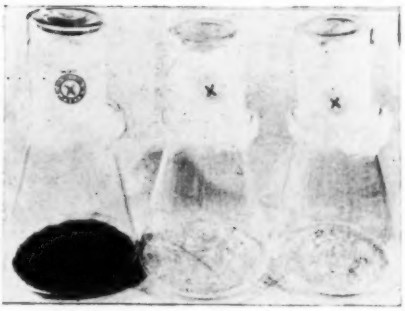
*——— and Fielding, A. H. .. "The use of *Aspergillus niger* (M) in the bio-assary of magnesium, copper, zinc and molybdenum in soils," *Ann. Rep. Long Ashton Res. Sta.*, 1947, 126–37.

'M' Strain

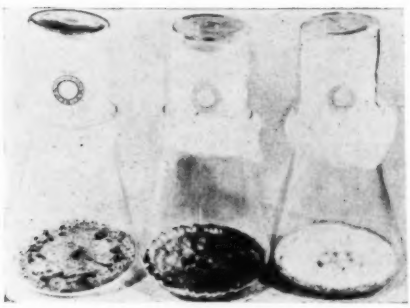


+all −all −Fe

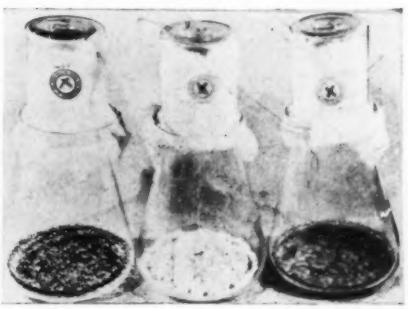
M.U.B.L. Strain



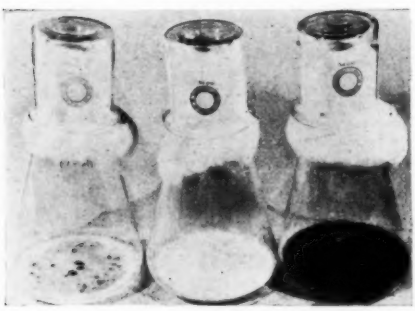
+all −all −Fe



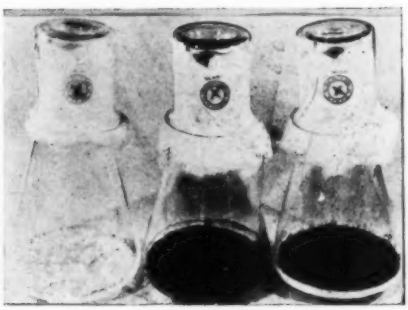
−Zn −Mn −Cu



−Zn −Mn −Cu



−all −Mo +all



−all +all −Mo

*Nicholas, D. J. D., and Fielding, A. H. "The use of *Aspergillus niger* for the determination of magnesium, copper, zinc and molybdenum available in soils to crop plants," Abstracts of *Proceedings 1st Internat. Congr. Biochem.*, Cambridge, Abs. No. 121/2, 1949, 302.

———— .. "Use of *Aspergillus niger* as a test organism for determining molybdenum available in soils to crop plants," *Nature*, 1950, **166**, 342.

———— .. "The use of *Aspergillus niger* (M) for the determination of magnesium, zinc, copper and molybdenum available in soil to crop plants," *J. hort. Sci.*, 1951, **26**, 125–47.

Steinberg, R. A. .. "Use of micro-organisms to determine essentiality of minor elements," *Soil Sci.*, 1945, **60**, 185–89.

* Not seen in original.

# COMPLETE ENUMERATION EXPERIMENT IN GROUNDNUT CROP FOR DEVELOPING THE OPTIMUM SHAPE AND SIZE OF SAMPLES FOR CROP WEATHER OBSERVATIONS

By A. K. Mallik, V. K. Subramanian and R. Venkatraman
(*Meteorological Office, Poona*)

Received July 27, 1953
(Communicated by Shri S. P. Venkiteshwaran, F.A.SC.)

## I. Introduction

Under the all-India Co-ordinated Crop-Weather Scheme, systematic observations on the growth, development and yield of crops and the meteorological conditions experienced by the crops are being recorded at a network of selected stations. The sampling techniques for estimating these growth features as well as the yield, in regard to the different crops of India were developed after actual preliminary investigation and trial by the Agricultural Meteorology Division at Poona. Results of a number of these experiments have already been published[1–9] while those of a few others will be published shortly.

The crop-weather observations are at present confined to Paddy, Wheat, Jowar, Sugarcane and Cotton. Recently, the Indian Central Oil Seeds Committee desired to extend the crop-weather observations to the groundnut crop also. In order to find out the most suitable size and shape of the sample to be used for recording the growth and yield of the groundnut crop, a complete enumeration experiment was undertaken. The results of this experiment are discussed in the present paper.

The experiment was conducted on the fields of the Agricultural College, Poona, the variety under investigation being the sub-erect type "Spanish Peanut". When the crop was ready for harvest, a net area containing 16 rows, each row being 72 feet long (1 foot apart) aggregating to $16 \times 72$ or 1,152 foot-lengths of row, of the crop was harvested foot by foot and the following observations, for each foot-length, were recorded:—

(1) Number of plants in foot-length;

(2) Number of branches in foot-length; and

(3) The yield of pods (gm.) from all the plants in foot-length.

For the purpose of analysis of the data, the net area was divided into four equal plots by imaginary lines drawn across the rows so that each plot had 16 rows, each row being 18 feet long, *i.e.*, in all 288 foot-lengths of row.

## 2. Analysis of Variance of the Data

The analysis of variance, for all the three characters under observation, is given in Table I below.

TABLE I

*Analysis of Variance*

| Crop feature | Variance | | | Total |
|---|---|---|---|---|
| | Between plots | Between rows | Within row (error) | |
| 1. No. of plants in foot-length .. .. | 0·44 | 1·28 | 0·66 | 0·69 |
| 2. Total number of branches in foot-length[4] .. | 82·13 | 54·85 | 30·29 | 31·70 |
| 3. Yield of pods from foot-length .. .. | 746 | 255 | 145 | 152 |
| Degrees of Freedom .. | 3 | 60 | 1088 | 1151 |

It will be seen from Table I that, for all the three characters, the variance 'between rows' is greater than that 'within row'; in other words the inter-row competition is greater than the intra-row competition. This suggests that samples spread across the rows will be more efficient than those spread along the same row.

## 3. Size and Shape of the Sample

As has been mentioned already, the ultimate unit for the observations was 1 foot-length of row. These ultimate units were then grouped in different combinations as explained below. Thus, for a sample of 6 foot-lengths, the following different arrangements of the ultimate units are possible.

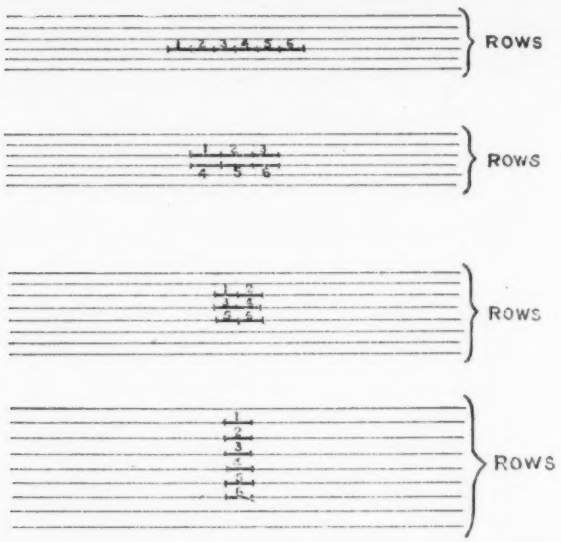
(i) All the 6 'one foot-lengths' in the same row.

(ii) '3 foot-lengths' in each of two adjacent rows.

(iii) '2 foot-lengths' in each of three adjacent rows.

(iv) 1 foot-length in each of six adjacent rows.

The above combinations are represented diagrammatically below.




However, it may be added here that as described earlier, the data from each plot consist of 16 rows, each row 18 feet long. This sets a limitation to the number of combinations that can be conveniently formed. For example, if combination number (iv) is tried the data relating to 12 rows only could be utilized (as each sample will be spread over 6 rows) and therefore the data for four rows of each plot will be left out of consideration. Hence only such combinations of the ultimate units which make it possible to include a major part of the data were considered. Thus, in the above example, combination (iv) was not included for analysis.

The following different shapes and sizes of the sample (composite groups) were considered.

The standard error of the mean for each sample $\sigma s/\sqrt{N}$ ($\sigma s$ = square root of variance between the ultimate units of the sample and N = number of ultimate units in the sample) were calculated and plotted against the size of the sample, *i.e.*, the number of foot-lengths in sample. Figs. 1, 2 and 3 show these curves for the three characters, *viz.*, number of plants, number of branches and yield respectively, for both, samples spread across the rows and samples spread along the rows. Values for square shapes in samples covering as many rows as foot-lengths in each row have not been plotted as only 3 such sample sizes, *i.e.*, $2\times2$, $3\times3$ and $4\times4$ are possible with the available data.

| No. of 1 foot-length included in the sample | No. of 1 foot-length of each row included in the sample | No. of rows over which the sample is spread |
|---|---|---|
| 2 | 2 | 1 |
| | 1 | 2 |
| 3 | 3 | 1 |
| | 1 | 3 |
| 4 | 4 | 1 |
| | 2 | 2 |
| | 1 | 4 |
| 6 | 6 | 1 |
| | 3 | 2 |
| | 2 | 3 |
| 8 | 8 | 1 |
| | 4 | 2 |
| | 2 | 4 |
| | 1 | 8 |
| 9 | 9 | 1 |
| | 3 | 3 |
| 16 | 16 | 1 |
| | 8 | 2 |
| | 4 | 4 |
| | 2 | 8 |
| | 1 | 16 |

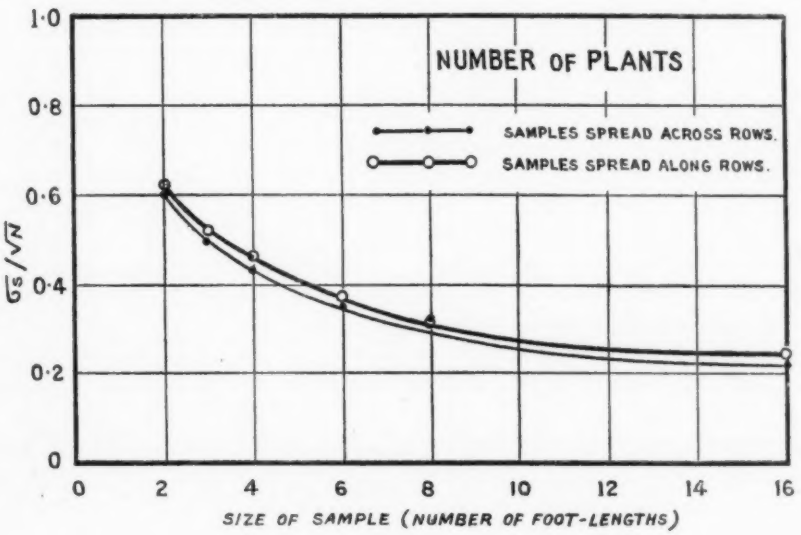



FIG. 1

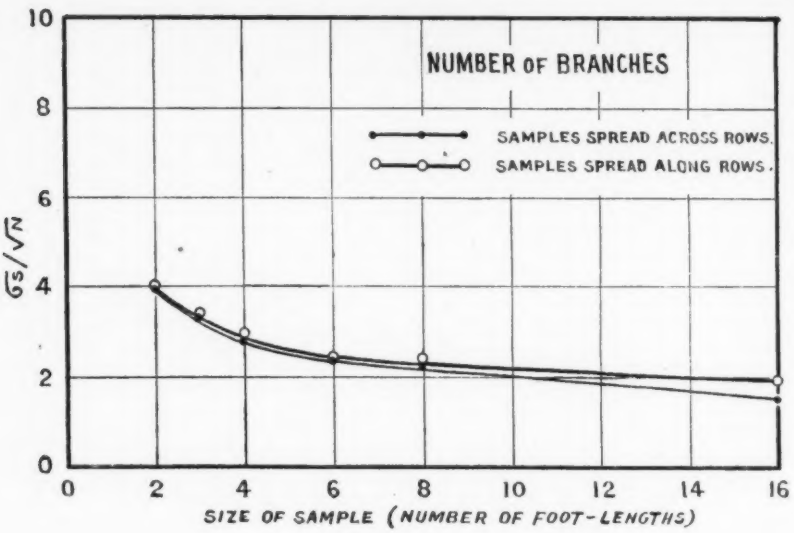

NUMBER OF BRANCHES
SAMPLES SPREAD ACROSS ROWS.
SAMPLES SPREAD ALONG ROWS.
$\sigma s/\sqrt{N}$
0
2
4
6
8
10
12
14
16
SIZE OF SAMPLE (NUMBER OF FOOT-LENGTHS)

FIG. 2

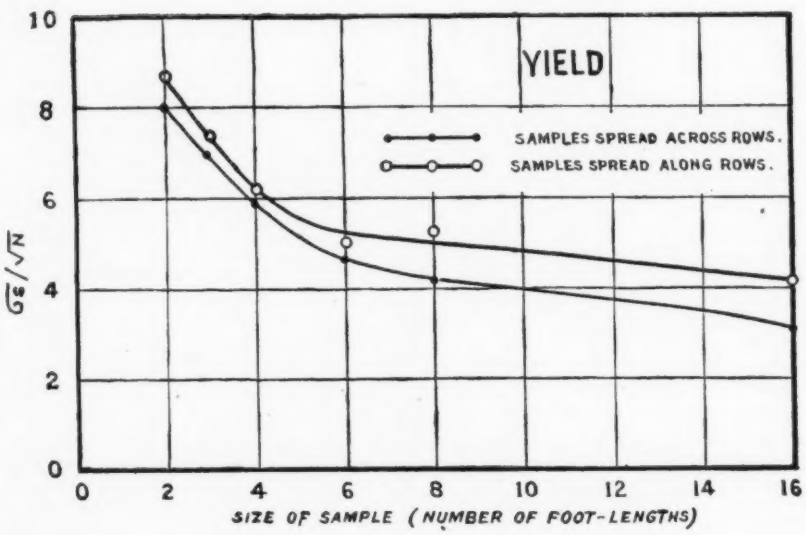

YIELD
SAMPLES SPREAD ACROSS ROWS.
SAMPLES SPREAD ALONG ROWS.
$\sigma s/\sqrt{N}$
0
2
4
6
8
10
12
14
16
SIZE OF SAMPLE (NUMBER OF FOOT-LENGTHS)

FIG. 3

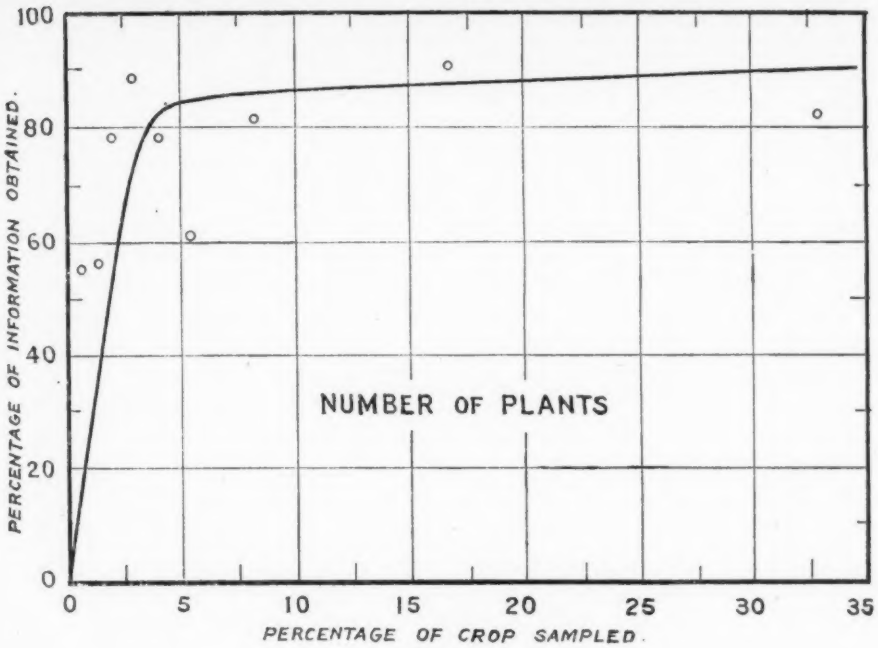

100
80
60
40
20
0
PERCENTAGE OF INFORMATION OBTAINED.
NUMBER OF PLANTS
0
5
10
15
20
25
30
35
PERCENTAGE OF CROP SAMPLED.

FIG. 4

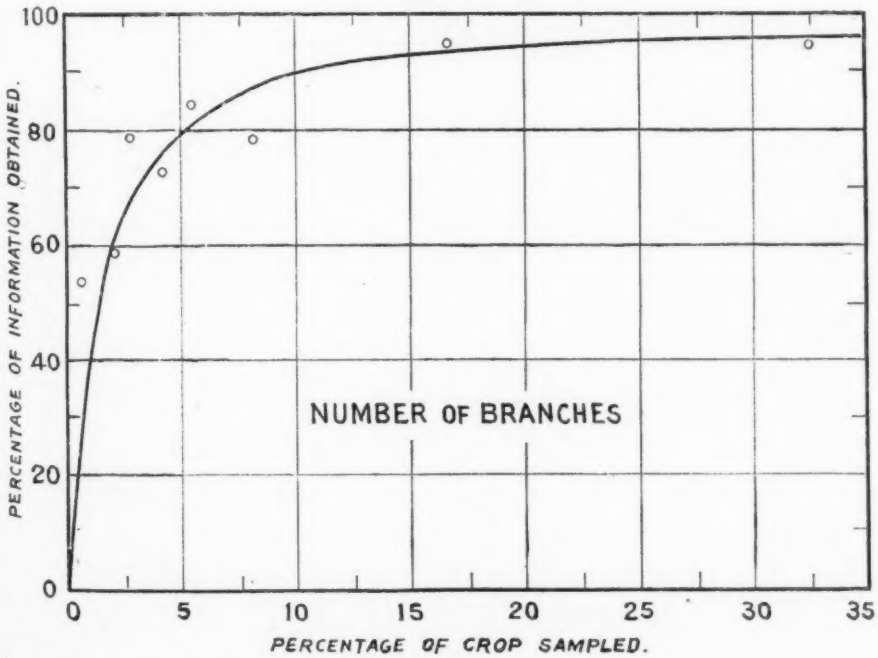

100
80
60
40
20
0
PERCENTAGE OF INFORMATION OBTAINED.
NUMBER OF BRANCHES
0
5
10
15
20
25
30
35
PERCENTAGE OF CROP SAMPLED.

FIG. 5

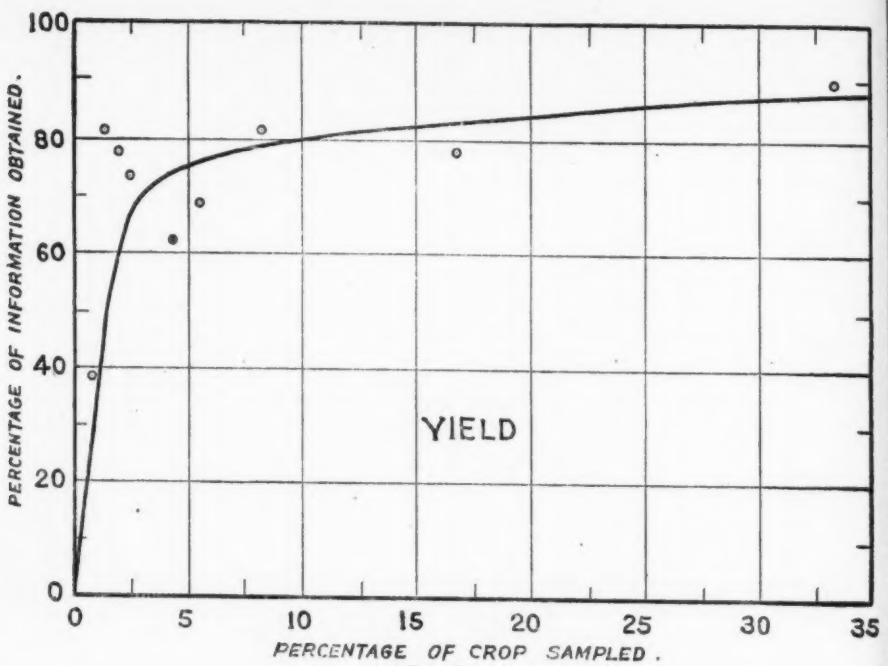
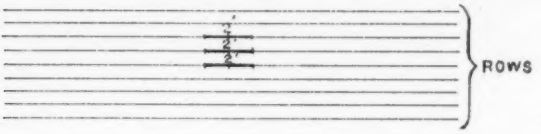



FIG. 6

It is seen from Figs. 1, 2 and 3 that for all the three characters under observation, $\sigma s/\sqrt{N}$ decreases as the size of the sample increases showing that the variability of the mean for the sample decreases with increase in the size of the sample. But the decrease is greater for the smaller sample sizes and as the size of the sample increases, this decrease in $\sigma s/\sqrt{N}$ becomes less and less. It appears from the curves that a sample of 6 foot-lengths will be quite suitable, for all practical purposes. It is also seen from Fig. 1 2 and 3, that for the same size of the sample, sample spread across the rows, *i.e.*, covering a larger number of rows, show less standard error of the mean than those spread along the rows, *i.e.*, including a smaller number of rows. This is true particularly for the yield. This is in agreement with the finding mentioned earlier that inter-row competition is found to be greater than intra-row competition.

It appears therefore that a sample composed of 6 foot-lengths including 2 foot-lengths in each of three adjacent rows (as shown in the diagram below) will be quite suitable for recording the crop-weather observations.




## 4. Percentage of the Crop to be Sampled

Having arrived at a suitable size and shape of the sampling unit to be used, it is necessary to find out what percentage of the crop should be sampled in order to obtain sufficient information. For this purpose, the percentage information obtained for different percentages of the crop sampled at random, were calculated using the method developed by Yates and Zacopanay[10]. The relationship between the percentages of the crop sampled and the percentages of information obtained for the number of plants, number of branches and the yield is shown graphically in Figs. 4, 5 and 6 respectively. It will be seen that the increase in the percentage information obtained is rapid till a critical value in the percentage of crop sampled is reached. Further increase in the percentage sampled increases the information obtained at a slower rate. This critical value for the percentage of crop sampled, appears to be:

(i) about 4% for number of plants (Fig. 4);

(ii) about 6% for number of branches (Fig. 5); and

(iii) about 5% for yield (Fig. 6).

From what has been explained above as well as from practical considerations, it is seen that for all the three characters under considerations, sampling 5% of the crop using a sampling unit consisting of 2 foot-lengths in each of three adjacent rows will be quite adequate for recording crop-weather observations in the suberect type of groundnut.

## 5. Acknowledgement

Thanks are due to the authorities of Agricultural College, Poona, for permitting these observations to be recorded on the crop raised by them. The authors also acknowledge with thanks the help given by Shri J. G. Griffiths in weighing the yield of pods and that of the other colleagues who helped in recording the observations in the field.

## REFERENCES


1. Mallik, A. K., Satakopan, V. and Gopal Rao, S. — *Ind. Jour. Agri. Sci.*, 1945, **15**, Pt. IV, 219–25.
2. Reports by the Director of Agricultural Meteorology.
3. Report on the Co-ordinated Crop-Weather Scheme, I.C.A.R., for the year 1948–49.
4. *Ibid.*, for the year 1949–50.
5. Report on the Co-ordinated Crop-Weather Scheme relating to sugarcane (sanctioned by the I.C.S.C.) for the year ending 31st March 1950.
6. *Ibid.*, for the year ending 31st March 1951.
7. *Ibid.*, for the year ending 31st March 1952.
8. Report on the Co-ordinated Crop-Weather Scheme, relating to cotton for the year ending 31st March 1950.
9. *Ibid.*, for the year ending 31st March 1951.
10. Sreenivasan, P. S. .. *Ind. Jour. Met. and Geoph.*, 1950, **1**, No. 4, 286–90.
11. Yates, F. and Zacopanay, I. .. *Jour. Agri. Sci.*, 1935, **25**, 545–77.

# ADDITIONS TO FUNGI OF MADRAS—XVI

By T. S. Ramakrishnan and N. V. Sundaram
(*Agricultural Research Institute, Coimbatore*)

Received November 27, 1953

*Synchytrium alysicarpi* sp. nov.

Galls numerous, on stem and leaves, reddish orange in colour, galls on stem swollen, sometimes irregularly lobed; on leaf amphigenous, becoming cupulate and whitish with age; hypnospores numerous, imbedded within hypertrophied cells and surrounded by proliferating tissue, sub-globose or oval, orange brown, thick-walled, wall differentiated into a thickened endospore surrounded by laminated thicker exospore, with granular contents, 53–130 × 50–98 $\mu$; sorus sub-globose or elliptical made up of several sporangia, orange yellow in colour, 90–218 × 82–150 $\mu$; sporangium thin-walled, with orange yellow contents, 25 × 22 $\mu$ (19–43 × 19–37), round or polygonal due to pressure.

Gallae plurimæ, in culmis atque foliis, rubro-aurantiacæ colore; gallæ in culmis tumidæ, nonnumquam irregulariter lobatæ; gallæ in foliis amphigenæ, evadentes cupulatæ atque albidæ ætate provecta; hypnosporæ plurimæ, inclusæ in cellulis hypertrophiatis atque circumdatæ texture proliferante, sub-globosæ vel ovales, aurantiaco-brunneæ, crassis parietibus, 53–130 × 50–98 $\mu$; sorus subglobosus vel ellipticus constans nonnullis sporangiis, aurantiaco-luteus colore, 90–218 × 82–150 $\mu$; sporangium parietibus tenuibus præditum, contentis aurantiaco-luteus, 25 × 22 $\mu$ (19–43 × 19–37), teres vel ob pressionem lateralem polygonale.

On living leaves and stem of *Alysicarpus vaginalis* DC. (Papilionatæ), Walayar (Malabar), 19-9-1953, N. V. Sundaram and A. Venkata Rao.

Galls are formed on the leaves and stem. Those formed on the stem are much bigger and sometimes confluent forming irregular fleshy masses. On the leaves the galls are smaller and in later stages become cupulate with one or more cups formed in each gall. The cups turn white in colour and are hairy at the margin. Numerous hypnospores and sporangia are present. The wall of the hypnospore can be differentiated into two regions, an inner brown endospore and an outer lighter coloured lamellated thickened portion. The measurements of the resting spores and sporangia and the peculiar formation of the galls indicate that the fungus under study is different from the other species recorded on the Leguminosæ.

*Phyllachora ventilaginis* sp. nov.

Spots yellowish brown, irregular, amphigenous, 2–5 mm. long and 1–3 mm. broad; stromata amphigenous, black, shiny, often reticulate, multiloculate, clypeate, occupying the entire thickness of the mesophyll; loculi 181–224 × 140–220 $\mu$, ostiolate; asci cylindric, hyaline, 8-spored, paraphysate, paraphyses rare, filiform, gelatinising, $78 \times 7 \cdot 5\,\mu$ (62–99 × 6–9); spores hyaline, one-celled, spindle-shaped, obliquely uniseriate, $12 \times 4 \cdot 5\,\mu$ (9–15 × 3–6).

Maculæ luteo-brunneæ, irregulares, amphigenæ, 2–5 mm. longæ, 1–3 mm. latæ; stromata amphigena, nigra, nitentia, sæpe reticulata, multiloculata, clypeata, totum mesophyllum occupantia; loculi 181–224 × 140–220 $\mu$, ostiolata; asci cylindrici, hyalini, octospori, paraphysati, paraphysibus paucis filiformibus, evadentes gelatinati, $78 \times 7 \cdot 5\,\mu$ (62–99 × 6–9); sporæ hyalinæ, unicellulatæ, fusiformes, oblique uniseriatæ, $12 \times 4 \cdot 5\,\mu$ (9–15 × 3–6).

On living leaves of *Ventilago maderaspatana*, Gærtn. (Rhamnaceæ), Kallar (Coimbatore), 21-7-1953, N. V. Sundaram.

The spots appear as black irregular, raised formations visible on both sides of the leaf. Small projections are evident on either surface of the shining stromata indicating the position of the ostioles of the young perithecia. *Phyllachora* has not been recorded on this host.

*Physalospora pyrenacanthæ* sp. nov.

Spots irregular, amphigenous, hypertrophied, raised on the upper surface, brown, 1–4 mm. across, studded with numerous black dot-like projections; perithecia sub-globose, crowded in the hypertrophied area, separate, opening predominantly on the upper surface, innate, reaching up to the middle of the mesophyll, uniloculate, ostiolate, 140–210 $\mu$ in diameter; asci clavate to cylindrical with rounded apex, hyaline, the wall slightly thickened at the apex, $120 \times 18\,\mu$ (93–150 × 16–21), paraphysate, paraphyses multicellular, hyaline; ascospores 8, irregularly biseriate, one celled, oblong, hyaline, $19 \times 9\,\mu$ (15–22 × 8–12).

Maculæ irregulares, amphigenæ, hypertrophiatæ, elevatæ in pagina superiore, brunneæ, 1–4 mm. diameter, punctata pluribus punctis nigris eminentibus; perithecia subglobosa, aggregata in area hypertrophiata, separata, ut plurimum dehiscentia in pagina superiore, innata, decurrentia usque in medium mesophyllum, uniloculata, 140–210 $\mu$ diameter; asci clavati vel cylindrici apice rotundato, hyalini, pariete aliquantum crasso ad apicem, $120 \times 18\,\mu$ (93–150 × 16–21), paraphysati, paraphysibus multicellularibus, hyalinis; ascosporæ octo, irregulariter biseriatæ, unicellulatæ, olbongæ, hyalinæ, $19 \times 9\,\mu$ (15–22 × 8–12).

On living leaves, stem and fruits of *Pyrenacantha volubilis* Hk. (Icacinaceæ), Kallar (Coimbatore), 17–7–1953, N. V. Sundaram.

The infection spots are prominent on the leaves and may occur isolated or a number of them coalesce together. The spots are yellowish brown in the beginning but later turn brown. The upper surface of the spot is raised and there is a corresponding depression on the lower surface. In some cases however both the upper and lower surfaces are raised. There is an increase in the number of layers of cells in the mesophyll in the affected region resulting in the thickening of the tissues to twice or thrice the normal thickness. The hyphæ are inter- and intra-cellular and are usually hyaline in the deeper tissues but those accumulating near the surface turn brown. The perithecia appear as minute, black raised dots in the hypertrophied region. They are isolated and sunk in the tissues of the mesophyll, The peridium consists of two to three layers of brown cells but towards the ostiole a thickened black clypeus like development is formed. The ascus is fairly stout with very short stalk. The ascus wall is thickened and glistening at the apex. The paraphyses are many, multicellular, often presenting a beaded appearance. The ascospores are irregularly arranged.

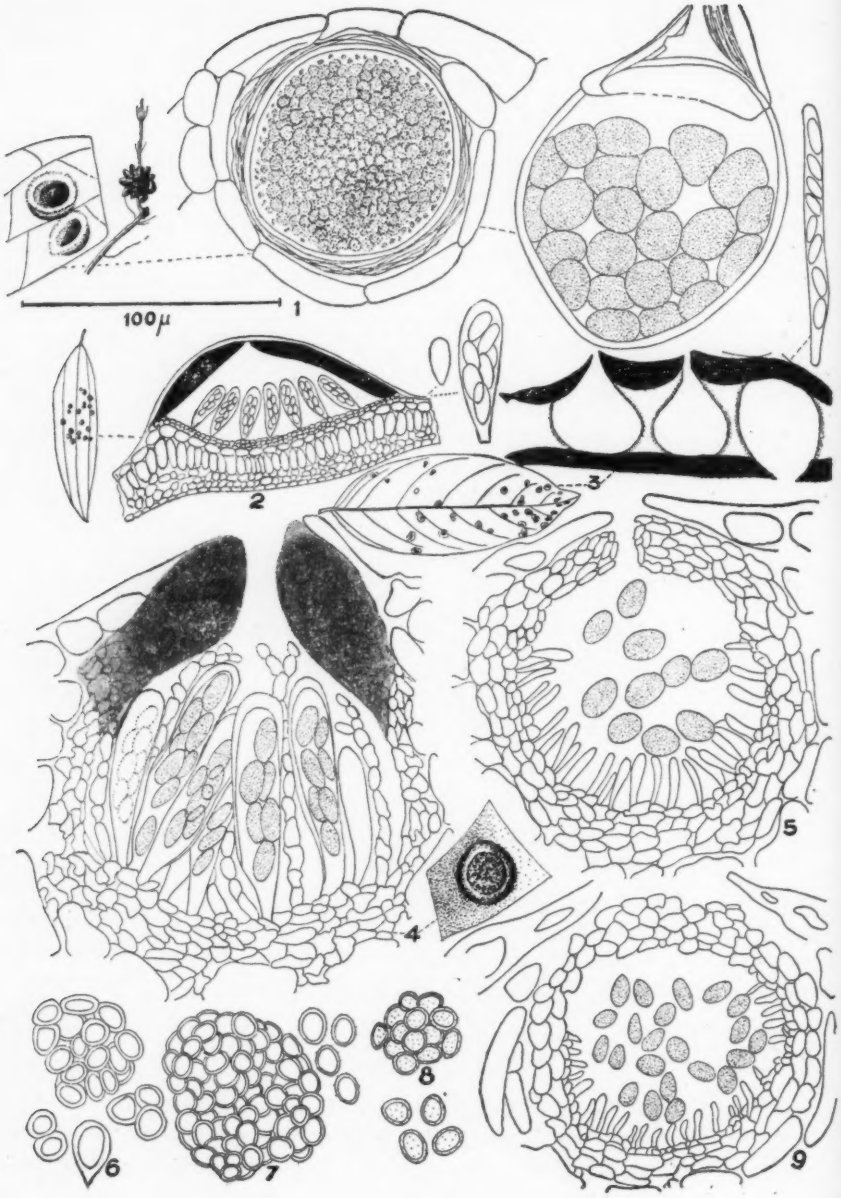
*Trabutia osbekiæ* sp. nov.

No definite spots; stromata amphigenous, black, shiny, raised, conical or mammiform, isolated or confluent forming small groups, subcuticular, ostiolate, with one or more locules; locules 120–169 × 38–70 $\mu$, paraphyses sparse; asci clavate, thin-walled, 8-spored, 47 × 12 $\mu$ (31–62 × 9–19); ascospores oblong, biseriate, thin-walled, hyaline, one-celled 16 × 6 $\mu$ (12–19 × 4·5–8).

Maculæ haud definitæ; stromata amphigena, nigra, nitentia, elevata, conica vel mammiformia, separata vel confluentia in catervas parvas, subcuticularia, ostiolata, uno vel pluribus loculis prædita; loculi 120–169 × 38–70 $\mu$, paraphyses raræ; asci clavati, tenui pariete præditi, hyalini, octospori, 47 × 12 $\mu$ (31–62 × 9–19); ascosporæ oblongæ, biseriatæ, tenuipariete præditæ, hyalinæ, unicellulatæ, 16 × 6 $\mu$ (12–19 × 4·5–8).

On living leaves of *Osbeckia octandra* DC. (Melastomaceæ), Wynaad (Malabar), 25–5–1953, T. S. Ramakrishnan.

Isolated or confluent stromata are formed on either surfaces of the leaf. Hyaline hyphæ penetrate into the mesophyll. The single celled nature of ascospores and the location of the stroma between the cuticle and the epidermis indicate that the fungus belongs to the genus *Trabutia*.




FIGS. 1–9

FIGS. 1–9. Fig. 1. *Synchytrium alysicarpi*: Galls on leaf (diagrammatic and enlarged), on petiole (natural size), hypnospores and sorus. Fig. 2. *Trabutia osbeckiæ*: Leaf showing the symptoms (natural size), section of perithecium (semi-diagrammatic), ascus and ascospore. Fig. 3. *Phyllachora ventilaginis*: Section of leaf showing the loculi (semi-diagrammatic) and ascus. Fig. 4. *Physalospora pyrenacanthae*: Section of perithecium and affected part of the leaf (enlarged). Fig. 5. *Macrophoma pyrenacanthae*: Section of pycnidium. Fig. 6. *Sorosporium tumefaciens*: Spore ball and spores. Fig. 7. *Tolyposporella sporoboli*: Spore balls and spores. Fig. 8. *Sorosporium cenchri*: Spore ball and spores. Fig. 9. *Macrophoma morindæ*: Section of pycnidium.

*Sorosporium tumefaciens* McAlpine

McAlpine, D., *Smuts of Australia*, Dept. Agric., Victoria, 1910, **184**.

Mundkur, B. B. and Thirumalachar, M. J., *Ustilaginales of India*, Commonwealth Mycological Institute, 1952, **56–58**.

Sori affecting the apices of the shoots and remaining concealed by the leaf-sheath, 15–25 mm. long covered by a greyish brown membrane which becomes lacerated at the apex, often recognised by protruding, dark, twisted columella; sporemass black, granular, surrounding the well-developed dark, central columella 15–20 mm. long, spore balls of various shapes, 43–115 $\times$ 31–68 $\mu$, black, opaque, permanent, composed of up to 160 spores; spores subglobose, verrucose on the free surface, dark brown in colour, 11·2 $\times$ 9·8 $\mu$ (8–14 $\times$ 7–12·5).

On shoots of *Chryosopgon montanus* Trin. (Gramineæ), Bhavanisagar (Coimbatore), 23–7–1953, Daniel Sundararaj.

This smut affects the culms and the sorus is mostly hidden in the leaf-sheath. The spore balls are fairly firm and the spores do not easily separate.

*Sorosporium cenchri* (Bref.) Zundel

Zundel, G. L. I., *Bothalia*, 1938, **3**, 283–330.

Smut ovaricolous, infecting all the spikelets in the inflorescence; sorus covered by false membrane which ruptures at the apex exposing the dark brown granular contents; a prominent central columella is present, spore balls evanescent; spores subglobose, brown, wall pitted, 11 $\times$ 10 $\mu$ (8–13 $\times$ 7–11).

On the inflorescences of *Cenchrus ciliaris* L. and *C. setigerus* Vahl. (Gramineæ), Coimbatore, 20–9–1951, N. V. Sundaram.

This smut has been recorded from Coimbatore and Kovilpatty. In some years a high incidence of this smut has been observed. Infection is systemic and all the ears of a plant are affected and in an ear all the spikelets are involved. Inoculation experiments have shown that the infection

is seed borne. Plants raised from artificially infected seeds were found affected by the smut while the controls were quite healthy.

*Tolyposporella sporoboli* Jackson

Whetzel, H. H. and Kern, F. D., *Mycologia*, **18**, 122, 1926.

Sori foliicolus, forming black and raised circular or elongated spots measuring upto 2 mm. in length, scattered or confluent, rupturing at a later stage; spore balls variable, indefinite, dark brown, 30–140 $\mu$; spores dark reddish brown, subglobose to oblong, often angular, sometimes occurring in pairs, outer wall drawn out to a point at one end in some cases, 15·4 $\times$ 11·2 $\mu$ (11·2 — 16·8 $\times$ 9·8 — 14), smooth and thickened upto 3 $\mu$.

On living leaves of *Sporobolus wallichii* Munro (Gramineæ), Walayar (Malabar), 19–9–1953, N. V. Sundaram and A. Venkata Rao.

This smut closely resembles *T. sporoboli* recorded from Porto Rico and is identified as such. This genus of smut has not been recorded on *Sporobolus* in India.

*Macrophoma morindæ* sp. nov.

Spots amphigenous, irregular, whitish grey, surrounded by a brown margin; pycnidia amphigenous, sometimes arranged in lines along the veins as black dots, immersed in the tissues, erumpent, ostiolate, 93–143 $\times$ 66–124 $\mu$; pycnidiospores oblong to elliptical, hyaline, pedicellate, one-celled, with granular contents, 10 $\times$ 7 $\mu$ (7–14 $\times$ 5·5–8·5).

Maculæ amphigenæ, irregulares, albido-griseæ, circumdatæ margine brunneo; pycnidia amphigena, nonnumquam disposita in lineas ad nervos ut puncta nigra, immersa in textus, erumpentia, ostiolata, 93–143 $\times$ 66–124 $\mu$; pycnidiosporæ oblongæ vel ellipticæ, hyalinæ, pedicellatæ, unicellulatæ, contentis granularibus, 10 $\times$ 7 $\mu$ (7–14 $\times$ 5·5–8·5).

On living leaves of *Morinda tinctoria* Roxb. (Rubiaceæ), Coimbatore, 21–9–1953, N. V. Sundaram.

The spots are conspicuous. The pycnidiospores are often embedded in mucilaginous substance.

*Macrophoma pyrenacanthæ* sp. nov.

Pycnidia occurring in hypertrophied spots, sparse or crowded, amphigenous, ostiolate, dark brown, oval to subglobose, 120–210 $\mu$ in diameter; pycnidiospores oblong, hyaline, pedicellate, one-celled with granular contents, 16 $\times$ 9 $\mu$ (12–22 $\times$ 6–12).

Pycnidia in locis hypertrophiatis, sparsa vel aggregata, amphigena, ostiolata, fusce brunnea, ovata vel subglobosa, 120–210 $\mu$ in diameter; pycnidiosporæ oblongæ, hyalinæ, pedicellatæ, unicellulatæ, contentis granularibus, $16 \times 9\,\mu$ (12–22 × 6–12).

On living leaves of *Pyrenacantha volubilis* Hk. (Icacinaceæ), Kallar (Coimbatore), 17-7-1953, N. V. Sundaram.

The pycnidia are often found associated with the perithecia of *Physalospora pyrenacanthæ*.

We are indebted to Rev. Fr. H. Santhapau for the Latin translation. We express our thanks to Dr. Biswas of the India Botanical Gardens, Calcutta, and to the Systematic Botanist and Professor of Botany, Agricultural College, Coimbatore, for their help in the identification of some of the host plants.

1040-54 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent
and Published by The Indian Academy of Sciences, Bangalore

# GROWTH STUDIES IN *SACCHARUM OFFICINARUM*

## VII. Number of Stalks in the Clump in Relation to Weight of Plant, Length of Millable Stalk and Quality Ratio of the Crop

By P. C. Raheja and G. S. Sekhon

(*Division of Agronomy, Indian Agricultural Research Institute, New Delhi*)

Received February 13, 1953

(Communicated by Dr. R. S. Varma, F.A.SC.)

### I. General

The study of number of canes in the clump in Sugarcane has received very considerable attention of research workers. Stubbs as early as 1900 stated that the variety which suckered the most profusely and gave the largest number of mature stalks to the plant should receive preference in cultivation. He emphasised the necessity of obtaining suckers as early as possible, for, the early formed canes conduced to greater yield and sugar in cane crop. Barber (1919) studied the problem from the aspect of morphology of cane. Rodrigues (1920) corroborated the conclusions of Stubbs (*loc. cit.*). According to Quintus (1923) as number of canes in the clump increased, the weight of canes also increased. The girth and length had a subordinate influence on the cane weight of the plant. Das (1931) compiled records of volumetric growth of cane and studied them in relation to quality ratio of the crop raised at Ewa plantation in Hawaii. From these data he observed that, " Other conditions being equal we are likely to get better juice from a field whose average ' Stalkage ' is lower. In other words we must promote early suckering and once having obtained the suckers we should try to prevent new shoot growth in the next season." Panje (1934) observed that early tillering had a very favourable influence on ultimate harvest and cane quality. Arceneaux (1935 and 1939) noted that compensating ability of the crop in respect of tillering, to cover up gaps in cane rows, is a varietal character. Variety Co 290 exhibited highest compensating growth and POJ 234 the least. Previous investigation in the series (Raheja, 1946; Raheja and Singh, 1952; Raheja, 1952) have indicated a highly significant correlation between relative growth rate and sucrose content in juice. Most of the crop at harvest consisted of mother shoots of the second order. Late formed shoots principally composed of third order shoots generally remained immature and died prematurely. The degree of variability in tillering was less than in plant weight but was more than in stalk weight. Varieties exhibited highly significant

linear relationship between weight per plant and number of canes per clump. There was little interdependence between number of canes in the clump and mean weight per tiller. Shoots of various orders of emergence indicated high consistency in their homogeneity in the characters of mean weight of stalk, length of stalk and mean weight per cm. stalk length.

## II. EXPERIMENTAL

In a four factor experiment consisting of *varieties* (2) × *depths of cultivation* (3) × *nitrogen levels* (3) × *phosphate levels* (2), was laid out in a split plot design, with the first factor as depth of cultivation which formed the main plot treatments. The treatments in the experiment are shown as under:—

TABLE I

*Nature of treatments in the experiment*

| Main plot treatments. Depth of Cultivation | Sub-plot treatments | | |
|---|---|---|---|
| | Varieties | Nitrogen levels per acre | Phosphate levels per acre |
| Co-Cultivation 3–4 in. deep with *desi* plough .. | $V_1$–Co 312 | $N_0$–0 lb. (Control) | $P_1$–0 lb. |
| $C_1$-Ploughing 6 in. deep; discing and grubbing | $V_2$–Co 453 | $N_1$–80 lb. | $P_2$–80 lb. |
| $C_2$-Sub soiling 10 in. deep ; discing grubbing .. | .. | $N_2$–160 lb. | .. |

According to normal practice three-budded setts were planted.

The experiment had three replications, each replication consisting of *thirty-six* plots. From every plot, *five* clumps at random were selected, one in each row, with the aid of random numbers (Fisher and Yates, 1938), in the month of December. The clump in each case consisted of the mother shoot and the suckers arising from it. This was ensured in every case, if necessary, by exposing the underground parts. In February such five clumps from every plot were selected from all the three replications. The stalk were cut flush to the ground level. The data recorded on each of the clumps consisted of number of canes, cane weight per plant of stripped and unstripped canes in December and of stripped canes in February, and their juice analysis. These data were statistically analysed. Total correlation coefficients and partial correlation coefficients and regression coefficients were worked out (Fisher, 1951). These have been given and interpreted in the succeeding text.

## III. Analysis of Observations

1. *Relationship between the number of canes and average weight per plant.*—In a preceding study (Raheja and Singh, 1952) it had been observed that the correlation values between number of canes and the weight of the millable stalks of the clump are very highly significant. Observations now were recorded on the whole weight of the clump, including foliage, to see how far the values of correlation coefficient varied from the correlation between number of canes and weight of millable stalks per plant. These are given in Table II below:—

Table II

*Coefficient of correlation and regression coefficient between number of canes and clump weight with and without foliage irrespective of treatment difference*

| Month of study | Character | Coefficient of correlation | | |
|---|---|---|---|---|
| | | Calculated | Theoretical (100 d.f.) 5% | 1% |
| December .. | 1 No. of canes per plump and total weight | ±0·758 | 0·1946 | 0·2540 |
| | 2 No. of canes per clump and stripped weight | ±0·793 | | |
| February .. | 1 No. of canes per clump and stripped cane weight | ±0·6108 | 0·1946 | 0·2540 |

It is obvious from the comparative values of correlation coefficients for December series that both the values were very high and significant at $P = 0·01$. The value for February series was also significant at 1 per cent. level of significance. In December, therefore, the weight of the clump and number of canes in the clump indicated a stronger relationship than in February. Besides, the results have also indicated that the total clump weight including foliage is as good a criterion of judging performance of a treatment as the number of canes in any series.

The analysis of February data was carried a stage further and correlation coefficients and regression coefficients for the doses of nitrogen and varieties were worked out separately. These are given in Table III.

In spite of the wide variation indicated the correlation coefficients in all cases were very highly significant. The regression coefficients did not show significant differences amongst the treatments.

In general, then, application of increasing doses of nitrogen increased the clump weight, by increasing the weight per cane. In variety Co 453

TABLE III

*Correlation coefficient and regression coefficient between number of canes and weight of plants for three levels of nitrogen and two varieties*

| Treatments | Correlation Coefficient | | | Regression coefficient | | |
|---|---|---|---|---|---|---|
| | Calculated | Theoretical 5% | 1% | 'b' value | S.E. of 'b' | 't' value |
| Nitrogen levels | | (34 d.f.) | | | | |
| $N_0$ .. | +0·526 | 0·324 | 0·418 | +0·699 | ±0·194 | 3·60* |
| $N_1$ .. | +0·610 | | | +0·733 | +0·163 | 4·50† |
| $N_2$ .. | +0·686 | | | +0·911 | +0·166 | 5·49‡ |
| Varieties | | (52 d.f.) | | | | |
| Co 453 .. | +0·825 | 0·273 | 0·354 | +1·704 | +0·161 | 10·58† |
| Co 312 .. | +9·896 | | | +1·249 | +0·085 | 14·70† |

* Indicates Sig. at 5% † Indicates Sig. at 1% ‡ Indicates Sig. at 0·1%

the increase in weight of every cane in the clump was relatively greater than in variety Co 312 which is apparent from the coefficient of regression values of + 1·704 and + 1·249 respectively.

2. *Relation between length of main stalk and clump weight.*—In a previous investigation (Raheja and Singh, 1954) it has been observed that the differences in the length of shoots arising out of the clumps at successive intervals does not vary significantly. In consequence thereof in this investigation the length of main shoot as an index of mean of cane length in a clump was measured for determining the relationship between changes in mean length of stalk and the clump weight. The summarised relationships between length of main shoot and clump weight are given in Table IV.

The analysis has indicated that, in general, the value of the correlation between mean length of main stalk and clump weight is very highly significant, the value $r = 0{\cdot}40$ ($P = 0{\cdot}01$). This indicates that the stalk length definitely influences the weight of the clump.

The nitrogen levels manifested wide difference in the values of the coefficients of correlation between height of the main stalk and the clump weight. The correlation was very strong between length of main stalk, as an indicator of mean stalk length, and the clump weight, the value $r = 0{\cdot}6129$ being significant at 1 per cent. level of significance. The values of the correlation were lower in the case of $N_1$ and $N_2$ treatments. These values were significant at 5 per cent. only.

TABLE IV

*Correlation coefficient and regresssion coefficient between average length of main stalk in the clump and clump weight*

| Treatments | Correlation Coefficient | | | Regression coefficient | | |
|---|---|---|---|---|---|---|
| | Calculated | Theoretical 5% | 1% | 'b' value | S.E. 'b' | 't' value |
| General .. | +0·40 | 0·1946 | 0·2540 | | | |
| Nitrogen doses | | (34 d.f.) | | | | |
| $N_0$ .. | +0·6129 | | | +0·0441 | ±0·0097 | 4·53* |
| $N_1$ .. | +0·3414 | 0·3246 | 0·4182 | +0·0265 | ±0·0125 | 2·12† |
| $N_2$ .. | +0·3897 | | | +0·0373 | +0·0151 | 2·46† |
| Varieties | | (52 d.f.) | | | | |
| Co 453 .. | +0·4072 | | | +0·0415 | ±0·0129 | 3·21* |
| Co 312 .. | +0·6132 | | | +0·0677 | +0·0120 | 5·60‡ |

* Indicates Sig. at 1% † Indicates Sig. at 5% ‡ Indicates Sig. at 0·1%

From this it appears plausible to presume, that by top dressing Cane Crop with nitrogen the mean weight of the clump increases relatively more than the length of the main stalk. In other words the degree of association between stalk length and clump weight decreased with the application of increasing doses of nitrogen to the cane crop, which is obvious from the lower regression values, for $N_1$ and $N_2$ treatments significant at 5 per cent. compared to regression value of $N_2$ treatment which was significant at 1 per cent. level of significance.

The value of the correlation coefficient between clump weight and length of main stalk for variety Co 312 was higher than Co 453. The values of regression coefficient for Co 453 was significant at 1 per cent. and of Co 312 significant at 0·1 per cent. level of significance. From this it may reasonably be concluded that the clump weight in Co 312 is more closely associated with mean length of stalk than in Co 453 variety. There is some other factor in the latter variety which influences this relationship in an appreciable manner.

3. *Relative influence of number of canes on the length of main stalk.*— Coefficient of correlation between number of canes per plant and length of main stalk was worked out for pooled data from all the observations recorded in February, irrespective of treatments.

TABLE V

*Coefficient of correlation between number of canes per plant and length of the main stalk*

| Characters | Coefficient of correlation | | |
|---|---|---|---|
| | Calculated | Theoretical 5% | 1% |
| No. of canes per clump and length of main stalk | −0·0168 | 0·1946 | 0·1638 |

Obviously the number of canes per clump and average length of main stalk in the clump are independent variables. The negative value of *r* indicates that with the increase in the tillering of the crop there is every probability of decrease in average length of main stalk in the clump.

4. *Interrelationship amongst the factors plant weight, number of stalks and main stalk length.*—In the preceding section it has been shown that correlations exist between (1) number of stalks per clump, and weight of clump, (2) number of stalks per clump and length of main stalk and (3) length of main stalk and weight of clump. In other words the three factors are interrelated and one influences the dimensions of the other two. To remove the effect of third variate and improve the precision of correlation partial correlations were worked out. The values of total and partial correlations are summarised in Table VI of the text.

TABLE VI

*Total and partial correlations for the characters of clump weight, number of stalks per clump and length of main stalk*

| Character | Correlation coefficients | |
|---|---|---|
| 1 | (Total 106 d.f.) 2 | Partial (105 d.f.) 3 |
| 1 No. of stalks per clump (1) and clump weight (2) .. | $r1, 2 = +0·6108$ | $r1, 2, 3 = +0·8061$ |
| 2 No. of cane per clump (1) and length of main stalk (3) | $r1, 3 = -0·1660$ | $r1, 3, 2 = -0·0763$ |
| 3 Length of main stalk (3) and clump weight (2) .. | $r3, 2 = +0·4896$ | $r3, 2, 1 = +0·7586$ |

The values of the partial correlations in the three combinations is higher than the total correlations. However, the order of effect is somewhat diffrent. The total correlation between number of canes and length of main stalk was low but partial correlation was very high when the effect of third

variate was equalised, which means that the number of stalks besides influencing the clump weight also have a negative effect on stalk length. As the stalk number increase there is tendency for stalk length to be reduced. The partial correlation between number of stalks in the clump and clump weight indicates that in the event of no change in the height of the main stalk the weight of clump and stalk number have a strong relationship between themselves. Similarly in the absence of any change in stalks' number the factors of length of stalk and clump weight are highly correlated. Considering all the partial correlations together the value is the highest between number of stalks per clump ($r = 0{\cdot}8061$) and clump weight followed by length of stalk and clump weight ($r = 0{\cdot}7586$) and negative correlation coefficient between length of main stalk and number of stalks per clump ($r = -0{\cdot}6763$) respectively. Evidently the increase in the length of stalk is a complementry factor to tillering (number of stalks per clump) in the increase in weight of clump and that tillering is of greater importance in improving plant weight than length of stalk.

5. *Number of stalks and sucrose relationship.*—The ultimate value of the cane crop is in its sugar content. It is, therefore, important to determine the effect of variation in the number of stalks per plant (tillering) upon sucrose per cent. in juice. This worked out relationship is shown in Table VII.

TABLE VII

*General coefficient of correlation between number of stalks per clump and sucrose per cent. in juice*

| Month | Characters | Correlation coefficient | | |
|---|---|---|---|---|
| | | Calculated | Theoretical 5% | 1% |
| December .. | No. of stalks per clump and sucrose per cent. in juice | −0·349 | 0·1946 | 0·2540 |
| February .. | Do | −0·396 | 0·1946 | 0·2540 |

It is evident that the nature of relationship did not alter whether the juice analysis was carried in December or February. With increase in tillering there occurred a significant fall ($> P = 0{\cdot}01$) in the sucrose content in cane. Regression equations were worked out and are given as under.

December series .. $Y = 13{\cdot}359 - 0{\cdot}174\times$

February series .. $Y = 16{\cdot}316 - 0{\cdot}146\times$

The relationship appears to be linear.

Since the difference in the amount of nitrogen application is known to materially influence the number of stalks per clump and sucrose in the cane, '*r*' and '*b*' values for the different nitrogen levels were worked out for February data between number of stalks per clump and sucrose in juice of the crop. These values have been tabulated as under:—

TABLE VIII

*Coefficient of correlation and coefficient of regression between number of stalks per clump and sucrose for various nitrogen levels*

| Treatments | Coefficient correlation | | | Regression Coefficient | | |
|---|---|---|---|---|---|---|
| | Calculated | Theoretical (34 d.f.) 5% | 1% | '*b*' value | S.E. of '*b*' | '*t*' value |
| $N_0$ .. | −0·647 | 0·324 | 0·418 | −0·211 | ±0·01353 | 15·60 *** |
| $N_1$ .. | −0·278 | | | −0·114 | ±0·02136 | 5·33 *** |
| $N_2$ .. | −0·330 | | | −0·113 | +0·0176 | 6·41 *** |

It is significant to note that the negative relationship between number of stalks per clump and sucrose content became weaker by application of nitrogen to the crop. The value was significant for $N_0$ at 1 per cent., for $N_1$ at 10 per cent. and $N_2$ at 5 per cent. In other words with the increase in tillering the sucrose per cent. in juice decreased, but comparatively more by nitrogen fertilization. From the point of view of cane culture this result is rather very interesting and its implications have been discussed later in the text.

6. *Number of stalks per clump in relation to purity of juice.*—The purity of juice usually does not follow the same course as the increase in the sucrose content of the cane crop. There is always a lag in the improvement in purity as there is increase in sucrose in juice. With this observation in view correlation coefficients were worked out between number of stalks per clump and purity per cent. juice for December and February series.

TABLE IX

*Coefficient of correlation between number of stalks per clump and purity per cent.*

| Month | Correlation coefficient | '*t*' value |
|---|---|---|
| December .. | −0·166 | 2·25* |
| February .. | −0·022 | 2·27 |

* Indicates significance at 5%.

A negative correlation between number of stalks per clump and purity per cent. of the expressed juice was indicated at both the periods. The relationship strong in December became weaker and weaker as the crop matured, the correlation in December being significant at $P = 0{\cdot}05$ but not significant for February values.

## IV. Interpretation of Results

In order to study the results in their proper perspective, with the aid of respective regression equations, the values showing the interrelationship amongst tillering, plant weight, length of stalk and quality of juice, were worked out and are given as under.

### Table X

*Interrelationship amongst tillering, plant weight, length of stalk and quality ratio irrespective of treatments to crop*

| No. of canes per clump | Length of stalk (cm.) | Weight of clump (lb.) | Sucrose in juice % | Brix value % | Purity % |
|---|---|---|---|---|---|
| 3 | 216·9 | 5·08 | 15·88 | 18·35 | 86·53 |
| 4 | 213·4 | 5·90 | 15·73 | 18·19 | 86·50 |
| 5 | 209·9 | 6·71 | 15·59 | 18·02 | 86·48 |
| 6 | 206·3 | 7·52 | 15·44 | 17·86 | 86·46 |
| 7 | 202·0 | 8·33 | 15·29 | 17·69 | 86·43 |

Briefly this study brings out the conclusion that with the increase in tillering and consequent increase in plant weight, there is tendency for the length of millable stalks, sucrose, brix and purity to decrease. The purity is affected to a much less extent compared to sucrose or brix values of the crop.

In general, soon after germination, the foliage of the plant starts carbon assimilation. The primary assimilation product is hexose which is synthesised into sucrose and starch. Narain (1932) noticed sucrose to constitute 80 per cent. or more of the total sugar of the soluble carbohydrate in the Ivy leaf. He further stated, "The amount of different carbohydrates at any time present in the leaf under natural conditions may be regarded as the resultant of at least four processes involving carbohydrates which are proceeding simultaneously:—

(i) Formation of simple carbohydrates by photosynthesis and hydrolysis;

(ii) Condensation of the above into more complex sugars and starch for temporary storage;

(iii) Translocation of soluble carbohydrates, including those obtained from hydrolysis of insoluble reserves to be used in growth and metabolism; and

(iv) Breakdown of all or any one of the above carbohydrates for respiration".

During the active growth the sugars are respired at a high rate. They are also actively synthesised into complex carbohydrates such as cellulose, pectin, gum, etc. Narain (*loc. cit.*) observed that total sugars in *Hydra helix* increased from June to December in spite of interconversion of carbohydrates for respiration and growth. The accumulation occurred because the processes of respiration and growth slowed down more as compared to the rate of active carbon assimilation. The balance between reducing sugars, sucrose, starch, cellulose, etc., is determined chiefly by temperature. Low temperature favours accumulation of sucrose and glucose (Harvey, 1929). High temperature, but not supra-optimal, favours condensation of primary sugars into polysaccharides for purposes of growth.

In the initial stages, from April to June, when the temperatures are very high, mean maximum being about 100° F., the primary sugars resulting from photosynthesis in the cane plant are immediately utilised for respiration, increasing the mass of protoplasm, the formation of cell walls, lignins, fats, lipoids, etc. In the initial stages, therefore, tissue formation proceeds at a rapid rate. These tissues ultimately develop into under-ground branches forming tillers and millable stalks. Das (1936) had observed significant correlation between hydration and rate 'of growth. Nitrogen fertilization increased hydration of tissues which simultaneously kept up high rate of growth of the crop. This is supported by the results given in the text, which have shown that the correlation coefficient between number of stalks and clump weight was high for $N_1$ and $N_2$ treatments compared to $N_0$ treatment. Evidently nitrogen induced greater hydration of tissues and maintained a high rate of growth.

The protoplasmic activity is very much retarded by deficiency of water. A more or less inverse relationship between moisture content and sucrose in cane has been observed to exist (Hartt, 1935). Cells high in water content elaborate carbohydrates of more complex type (Das, 1936). Das further held the view, " The lower sucrose content in the younger plants may, therefore, be attributed to the higher hydration of the tissues, under which conditions accumulation of monosaccharides is more feasible than the disaccharide sucrose." [ Quoted from Cornelison and Cooper (1941).] Borden (1946)

later reported that as hydration of tissues increased upon nitrogen applications the accumulation of sucrose decreased. "In young plants hydration is greater and sucrose lower than in older plants. As the crop advances in age, the hydration decreases and sucrose increases" (Parthasarathy *et al.*, 1951). It is, therefore, that with the increase in number of canes per clump the sucrose decreases although weight per plant simultaneously increases.

While discussing variability in the characters of the stalks in the cane plant (Raheja and Singh, 1952) it had been observed that the values of the standard errors of the weight of stalk, and length of stalk and mean weight per unit length of stalk were comparatively low upto 5 tiller group but were more than double in the next five shoots in order of emergence. This was due to the fact that the latter group of stalks exhibited very wide variation in the stalk characters. In other words the number of immature stalks increased with the increase in tillering. Obviously the degree of hydration of such stalks as emerged later was high compared to earlier formed stalks. The late formed stalks were relatively in more active state of growth than earlier formed shoots and in them the sucrose accumulation was low. Generally then as the number of shoots increased the overall hydration of the plant was high and sucrose low in December. With the low temperatures prevailing in the succeeding months the accumulation of sucrose in all classes of stalks tended to increase so that effect of immature stalks was much less evident. It is, therefore, that the value of correlation coefficient between tillering and sucrose in February was low and not significant.

## V. Summary

A study of the interrelationship amongst the factors of number of stalks per clump, clump weight, stalk length and quality ratio was conducted in the agronomical trial *Depth of cultivation* (3)× *Varieties* (2)×*Nitrogen levels* (3)×*phosphate levels* (2). The characters were studied in sample plants selected at random. The results are given as under:—

1. The relationship between tillering and whole plant weight, including the foliage, was as high as the relationship between tillering and the weight of the millable stalks in the clump.

2. By application of nitrogen the increase in plant weight occurred much greater by even relatively less increase in the number of millable stalks. The effect of depth of cultivation treatments and phosphate levels did not indicate similar relationship.

3. The increase in weight per plant with a unit weight increase in the number of stalk in the clump was relatively more in variety Co 453 than with Co 312.

4. With the relative increase in main stalk length there was significant increase in the weight per clump. The degree of association decreased by application of nitrogen to the crop, as the influence of tillering became relatively more pronounced on clump weight.

5. The number of stalks per clump and millable stalk length were negatively correlated, the value of $r$ being not significant.

6. Partial correlations amongst the factors of mean length of main stalk, mean clump weight and number of stalks per clump indicated that increased stalk length is a complementary factor to tillering in increasing the clump weight, the contribution of stalk length being much less than of stalkage in the increase in weight of the clump.

7. There was significant negative association ($> P = 0{\cdot}01$) between stalkage and sucrose per cent. in juice. This association became weaker as the cane matured with the fall in temperature and decrease in the hydration of tissues.

8. The immaturity of the late formed stalks reduced the sucrose content in juice, but relative increase in weight of clump more than offset the loss in length of millable stalk or sucrose per cent. juice.

## VI. ACKNOWLEDGEMENTS

The facilities for conducting these investigations were provided by the Head of the Division of Agronomy. Mr. K. P. Misra, M.SC. (AGRI. BOT.), ASSOC. I.A.R.I., was associated with the agronomical experiment. Dr. P. N. Saxena and S. Daljit Singh assisted us in statistical analysis of the data. Grateful acknowledgements are due to all of them.

## VII. BIBLIOGRAPHY

1. Arceneaux, G. .. "A study of sugarcane stalkage groups under Louisiana conditions," *Proc. 5th Cong. Int. Soc. S.C. Tech. Brisbane*, 1935, 777–78.

2. ———— .. "Studies of gaps of sugarcane rows and their effect upon yield under Louisiana conditions," *U.S. Dept. Agric. Circ.*, 1939, **521**, 1–21.

3. Barber, C. A. .. "Studies in Indian Canes, IV. Tillering or Underground branching," *Memo. Deptt. Agric. Ind. Bot. Ser.*, 1919, **10** (2).

4. Borden, R. J. .. "A search for guidance in the nitrogen fertilization of the Sugarcane crop, III," *Haw. Pl. Rec.*, 1946, **50**, 161.

5. Cornelison, A. M. and Cooper, H. F. "Further studies in the nitrogen nutrition. Time-of-application-of-nitrogen test," *Ibid.*, 1941, **45**, 155.

6. Crowther, F. .. "Studies in growth analysis of the cotton plant in Sudan III. A comparison of plant development in the Sudan Gezira and Egypt," *Ann. Bot.* (*N.S.*), 1944, **8**, 30–31.

7. Das, U. K. .. "Weather and juice quality at Ewa", *Haw. Pl. Rec.*, 1931, **35** (1).

8. ——— .. "A new approach to the problem of juice quality," *Report 15th Ann. Meeting Assoc. Haw. Sugar Tech.*, 1936.

9. Fisher, R. A. .. *Statistical Methods for Research Workers*, Oliver & Boyd, London, 98, Great Russell Street, W.C., 1951.

10 Fisher and Yates, F. .. *Statistical Tables for Biological, Agricultural and Medical Research*, Oliver & Boyd, London, 1938.

11. Hartt, C. E. .. "Fluctuations of sugars in cane leaf blades during night and day," *Haw. Pl. Rec.*, 1935, **39**, 298.

12. Harvey, R. B. .. *Plant Physiological Chemistry*, The Century Co., New York and London, 1929.

13. Narain, R. .. "Carbohydrate metabolism in respiring leaves of *Hydra helix*," *Ph.D. Thesis*, Cambridge University, 1932.

14. Panje, R. R. .. "A study of shoot production of sugarcane in relation to millable harvest," *Proc. Ann. Con. Sugar Tech. Assoc., India*, 1943.

15. Parthasarathy, S. V., Rao, W. T. and Vaidyanathan, S. "Tissue moisture and its relation to sugarcane ripening," *Proc. First. Bienn. Conf. S.C. Res. Workers, Coimbatore*, 1951.

16. Quintus, R. A. .. *The Cultivation of Sugarcane in Java*, Norman Roger, London E.C. 3, 1923.

17. Raheja, P. C. .. "Growth studies in *Saccharum officinarum*, I. Varietal Series," *Ind. J. Agri. Sci.*, 1946, **16**, (2), 113.

18. ——— and Singh, D. .. "Growth studies in *Saccharum officinarum*, IV. Tillering in relation to cane weight," *Ibid.*, 1952, **22** (12).

19. ——— .. "Growth studies in *Saccharum officinarum*, V. On the varietal differences in the relationship between weight and tillering of plant," *Ibid.*, 1954, **24**.

20. ——— .. "Growth studies in *Saccharum Officinarum*, VI. Variability in the characters of stalks in the plant," *Ibid.*, 1954, **24**.

21. Rodrigues, H. J. .. "Relation between high sucrose in cane and suckering," *Planter and Sugar Manuf.*, 1928, **80**, 101.

22. Stubbs, W. C. .. *Cultivation of Sugarcane*, Norman Roger, London, 1900.

# STUDIES ON INDIAN ICHNEUMONIDAE (HYMENOPTERA)

## Sub-family: BANCHINÆ. Part I. Tribe GLYPTINI

By E. S. Narayanan, M.A., Ph.D. (Lond.), D.I.C., F.R.E.S., F.E.S.I., F.A.Sc.
and
Kundan Lal, M.Sc. (Hons.)
(*Indian Agricultural Research Institute, New Delhi*)

Received October 19, 1953

**Contents**

| | Page |
|---|---|
| Introduction— | 38 |
| Zoological Nomenclature .. .. .. .. .. | 39 |
| Acknowledgment .. .. .. .. .. .. | 39 |
| Arrangement .. .. .. .. .. .. .. | 39 |
| Present Position of Indian Banchinæ .. .. .. | 40 |
| Sub-Family Banchinæ .. .. .. .. .. .. | 40 |
| Key to Tribes of Banchinæ .. .. .. .. | 40 |
| Tribe Glyptini .. .. .. .. .. .. | 41 |

### Introduction

In the history of biological science it has been the experience of workers that to stimulate further research and make enduring advances in a particular aspect of a biological problem we must make use of the knowledge and methods that we already possess on a rational basis. This is more so in the field of pure entomology especially systematic entomology. Here the first requisite for making a first class contribution to our knowledge of a particular group or family is to have an up-to-date Catalogue of the Species. This enables the worker to have a clear idea of the relationship that species bear to one another. The Ichneumonidæ is a family of great economic importance in that the vast majority of Ichneumon flies are parasitic on Lepidoptera which include some of the most serious of all pests of agricultural crops, both in the field and in storage.

Very little or practically no work has been done in India since Morley (1913) wrote his *Fauna of British India, Hymenoptera*, Volume III, about four decades ago. Since Morley wrote his Fauna in 1913, taxonomic work has made such advances that even his work completed in the first decade of

the present century has become out of date as the whole family has been reclassified especially in U.S.A. and Britian with new sub-families, tribes, genera and species and with new synonymy. Many names have had to be redefined, as wrong names had been applied to many genera and species. It is unfortunate that workers on this family should still have to use this volume for their taxonomic research work. For research work on the taxonomic aspects of this family is both difficult and time-consuming because of its extremely scattered literature and its confused taxonomic state at the present moment. A good deal of preliminary ground-work is necessary if the contribution is to be enduring. But for the various catalogues and revisional papers published by eminent taxonomists from time to time throughout the world including valuable contributions to our knowledge of Indian Banchinæ, the excellent library facilities and the Ichneumonidæ collection available at the Indian Agricultural Research Institute, New Delhi, the preparation of this systematic paper would have been well nigh impossible. The references to various species have been carefully verified in most cases and the recent views of the specialists on the synonymy of the group have been adopted.

**Zoological Nomenclature**

International rules on Zoological Nomenclature as supplemented by opinions have formed the basis for this systematic work. One nomenclatorial change that we have adopted is to base all the supergeneric names on the oldest included genus. Thus sub-family Lissonotinæ, Townes (1944) is changed in Banchinæ Muesebeck, Knombein, Townes and others (1951).

**Acknowledgment**

Taxonomic work on this important parasitic family Ichneumonidæ has been made possible under a generous grant sanctioned by the Indian Council of Agricultural Research under the School of Entomology. We are indebted to Prof. Townes, author of *A Catalogue and Reclassification of Nearctic Ichneumonidæ* for his valuable suggestions and comments. We wish to thank Dr. Richards, Imperial College of Science and Technology, London, for going through the manuscript and making some valuable corrections. We wish to thank Dr. B. P. Pal, Director, Indian Agricultural Research Institute, for the keen interest he has taken in this piece of research work.

**Arrangement**

Super-specific classification and 'Trivial names' have been adopted from Muesebeck and others (1951) and described accordingly.

**Present Position of Indian Banchinæ**

Very little or practically no work has been done on Indian Banchinæ after Morley (1913). C. Morley (1913) treated Lissonotides and Banchides as only tribes of the sub-family Pimplinæ. In dealing with the Ichneumon flies grouped together in the sub-family Pimplinæ he offered a number of suggestions for a new arrangement as he had doubts whether all the members were closely related. The following quotation from his Fauna will amply bear out this statement. " That the Lissonotides have any close relationship with typical Pimplides I do not for a moment believe. Banchides are admittedly aberrant wherever placed.....". Cushman and Rohwer (1920) while dealing with Holarctic tribes of Ichneumon flies of the sub-family Pimplinæ (= Ichneumoninæ) said " Lissonotini is closely allied to Banchini as represented by *Exetastes* Grav. and its nearest allies. Within the sub-family as here treated its closest relative is Glyptini, the abdominal structure of the latter being the only real difference. These two tribes form a group not at all closely related to the rest of the sub-family, Pimplinæ." The Pimplides of Morley is most heterogeneous. Genera *Glypta* Grav., *Glypto-pimpla* Morl., *Apophua* Morl. and *Miophantus* Cam. are closely related to Lissonotini rather than to Pimplides. Besides this *Phytodiætus* Grav. and *Phytodiatoides* Morl. given as genera under tribe Lissonotini by Morley (1913) have been withdrawn from this tribe as they are more allied to Tryphoninæ. Townes (1944) has segregated Lissonotini as a distinct sub-family from Pimplinæ.

In the preparation of the Key to the tribes we have taken into consideration besides adult characters, other characters also like the structure of the larva where possible and nature of host relationship, etc.

Sub-family BANCHINÆ (= LISSONOTINÆ)

The tribes included in this sub-family are Banchini, Glyptini and Lissonotini. The members of this sub-family have their head transverse and not cubical as in Xoridides, the abdomen is evenly sculptured, usually finely punctate or smooth throughout and not at all impressed (except Glyptini) or tuberculate as in the Pimplides, the basal segment of abdomen though sometime slender is not subpetiolate as in Hemigastrides; ovipositor with dorsal notch at a short distance from apex. These are internal parasites of Lepidoptera.

**Key to Tribes of Banchinæ**

1. Tergites with oblique ferrows extending from basal middle to near apical margin—Glyptini.
   Tergites without olbique ferrows .. .. 2

2. Abdomen not impressed or tuberculate, usually finally punctate; at least thorax dorsally and propodeum distinctly sculptured; areolet triangular, or oblique and smaller, or wanting ....Lissonotini.

   Areolet very large and rhomboidal; abdomen smooth and nitidulous; thorax entire, metathorax short with no area—Banchini.

   This key is adopted from Morley (1913) and Cushman (1921).

Lissonotoidea (Fam.) Förster, *Verh. Naturh. ver. Preuss. Rheinl.*, **25**: 142, 166, (1868).

Lissonotinæ Dalla Torre, *Cat. Hymen.*, **3**: 487, (1901). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 429, (1944).

Banchinæ Muesebeck, Krombein, Townes and others *U.S. Dep. Agric., Monograph* 2: 306, (1951).

## Tribe 1. GLYPTINI

Due to the ovipositor with dorsal notch a short distance back from apex and terga with oblique furrows extending from basal middle to apical margin the genus *Glypta* Grav. as treated by Morley (1913) in Fauna British India has been raised to the rank of a tribe having *Glypta* Grav., *Glyptopimpla* Morl. and *Apophua* Morl. as its genera.

Glyptini Cushman and Rohwer, *Proc. U.S. nat. Mus.*, **57**: 393, (1920).

Townes, *Mem. Amer. ent. Soc.*, **11** (1): 424, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric., Monograph* 2: 306, (1951).

### **Glypta** Gravenhorst

*Glypta* Gravenhorst,, *Ichn. Eur.*, **3**: 3, (1829), (Type of genus: *G. teres* Grav.). Dalla Torre, *Cat. Hymen.*, **3**: 408, (1901-02). SchmiedeKnecht, *Genera Insect., fas.* 62: 47, (1907), Morley, *Fauna Brit. India, Hymen.*, **3**: 210, (1913)[1]. Dutt, *Mem. Dept. Agric. India, Ent.*, **8** (2): 16, (1923). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 429, (1944).

[1]Europe, North and South Africa, Malay Archipelago, Japan, North America, Chile, Himalayas.

*Conoblasta* Fœrster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 165, (1868).

*Diblastomorpha* Förster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 165, (1868).

*Hemiephialtes* Ashmead, *Proc. U.S. nat. Mus.*, **30**: 177, (1906).

*Zaglyptomorpha* Viereck, *Proc. U.S. nat. Mus.*, **46**: 385, (1913).

**evanescens** Ratzeburg

*Glypta evanescens* Ratzeburg, *Ichn. d. Forst.*, **2**: 103. Thompson, *Opusc. ent.*, **13**: 1933. ♂, ♀. *cf.* Bridgman, *Trans. ent. Soc.*, p. 436, (1889). Schmiede Knecht, *Genera Insect.*, *fas.* 62: 48, (1907). Morley, *Ichn. Brit.*, 3: 169, (1907).

**nigrina** Desvignes

*Glypta nigrina* Desvignes, *Cat. Brit. Ichneum.*, p. 74, (1856). Dalla Torre, *Cat. Hymen.*, 3: 413, (1901–02). Schmiede Knecht, *Genera Insect.*, *fas.* 62: 48, (1907). Morley, *Ichn. Brit.*, 3: 161, (1907). ♂, ♀; *Fauna Brit. India, Hymen.*, 3: 211, (1913). ♂, ♀;[1] Dutt, *Mem. Dep. Agric. India, Ent.*, 8 (2): 16, (1923).[2]

[1]Punjab: Simla; Sikkim: Rungarum; Darjeeling.
[2]Punjab: Murree

*Glypta flavipes* Desvignes, *Cat. Brit. Ichneum.*, p. 75, (1856). ♂.

*Glypta nursei* Cam., *J. Bombay nat. His. Soc.*, **14**: 425, (1902). ♂.

**tricarinata** Cameron

*Glypta tricarinata* Cameron, *Zeits. Hymen. Dip.*, p. 41, (1908). ♀. Morley, *Fauna Brit. India, Hymen.*, 3: 212, (1913). ♀[1].

[1]Sikkim.

**Glyptopimpla** Morley

*Glyptopimpla* Morley, *Fauna Brit. India, Hymen.*, 3: 209, (1913)

(Type of the genus: *Glyptopimpla prima* Morley).

**prima** Morley

*Glyptopimpla prima* Morley, *Fauna Brit. India, Hymen.*, **3**: 210, (1913). ♂.[1] [1]Sikkim

**Apophua** Morley

*Apophua* Morley, *Fauna Brit. India, Hymen.*, **3**: 213, (1913)[1] (Type of the genus: *Apophua metopiiformis* Morley, present designation). [1]Assam: Ceylon; Sikkim; Shanghai.

**carinata** Morley

*Apophua carinata* Morley, *Fauna Brit. India, Hymen.*, **3**: 215, (1913). ♀.[1] [1]Assam: Khasi Hills; Ceylon: Maskeliya.

**metopiiformis** Morley

*Apophua metopiiformis* Morley, *Fauna Brit. India, Hymen.*, **3**: 214, (1913), ♂.[1] [1]Sikkim.

# THE EFFECT OF PRAWN PROTEIN ON THE BONE OF MATURE RATS

By T. C. Appanna, M.Sc., Ph.D.
AND
S. C. Devadatta, D.Sc. (Edin.), F.A.Sc.
(*Christian Medical College, Vellore*)

Received October 22, 1953

The present study was undertaken in order to obtain data relative to the influence of the nutritional variations studied on the bones of normal limbs of mature rats. There is a wealth of knowledge pertaining to the effects of dietary deficiencies of minerals on the skeleton of young animals; and Sherman and co-workers (Sherman and Booher, 1939; Sherman and Sherman, 1938; Van Duyne *et al.*, 1941; Sherman *et al.*, 1941) have investigated extensively the beneficial effects of diets rich in calcium, on growth and calcium storage in young animals. While there is considerable information (Conner and Sherman, 1936; Osborne and Mendel, 1926; Quinn *et al.*, 1929) dealing with several phases of the influence of protein intake on skeletal growth of young rats, there appears to be little published knowledge with regard to the effect produced by protein deficient diets on the bones of *mature* animals. And also the finding (Armstrong, 1948) that severe restriction of protein content of the diet of mature rats produced marked effect on the humeri, suggested the present problem of the influence of different levels of prawn protein intake on the bones of such animals.

## Method

Diets containing respectively 5%, 10%, 12·5%, 15%, 17·5% and 20% of prawn protein given in Table I were prepared according to the method described by Armstrong (1948). Additional sucrose was added on an equal weight basis, as a replacement for protein in the 3 diets of lower protein content.

From a colony that was fed with a stock diet (Table II) well balanced as regards fat, carbohydrate, protein, minerals and vitamins, 60 albino mature rats were selected with weights between 240–50 gm. They were fed again on stock diet and carefully watched until their weights were within the range of 260–65 gm. when they were started on dietary regimen. The rats were distributed equally with regard to sex and were housed in cylindrical cages. Water was also supplied *ad libitum*.

TABLE I

*Composition in gm. per Kilogram of Diet*

| Diets | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Prawn Protein (Pulp) .. | 75 | 100 | 125 | 150 | 175 | 200 |
| Sucrose .. .. .. | 425 | 400 | 375 | 350 | 325 | 300 |
| Sucrose and Vitamins* .. | 280 | 280 | 280 | 280 | 280 | 280 |
| Sweet Oil .. .. | 169 | 169 | 169 | 169 | 169 | 169 |
| Salt Mixture (Osborne and Mendel) .. .. | 40 | 40 | 40 | 40 | 40 | 40 |
| Yeast .. .. .. | 10 | 10 | 10 | 10 | 10 | 10 |
| Sodium Chloride .. .. | 1 | 1 | 1 | 1 | 1 | 1 |

* Each 1,000 gm. of diet contained 2 mg. thiamine hydrochloride, 2 mg. pyridoxine hydrochloride 3 mg. riboflavin, and 4 mg. calcium pantothenate.

TABLE II

Stock Diet

| | | | |
|---|---|---|---|
| Wheat flour .. .. | 60 gm. | Sodium Chloride .. | 0·50 gm. |
| Gram flour .. .. | 20 „ | Calcium Carbonate .. | 1·50 „ |
| Ankoria Baby Food .. | 5 „ | Milk .. .. .. | 60 cc. |
| Sweet Oil .. .. | 5 „ | Sodium Phosphate .. | 3·60 gm. |
| Meat Paste .. .. | 2 „ | Ascorbic Acid .. | 6 mgm. |

The six diets, *viz.*, 5, 10, 12·5, 15, 17·5 and 20% diets of Sode (*Parapæniopsis sculptilus*) were fed to the correspondingly numbered groups of animals, each group containing 10 animals, for a period of 30 days. The animals were sacrificed and after prolonged boiling of the fore-limbs in water, the humeri were dissected free of soft tissue. The bones were dried and rendered fat-free by continuous extraction in a Soxhlet apparatus for 48 hours with a mixture of equal parts of alcohol and ether. Again the

bones were dried and weighed, the length of the bones was determined to the nearest 0·1 mm. with a vernier micrometer. Finally they were ashed to constant weight at 700° C. and their weight recorded. The ash of the humeri of the individual animal was dissolved in hydrochloric acid and analysed for calcium and phosphorus. The calcium determinations were made by the method of Clark and Collip (1925) and the phosphorus analyses by the method of Fiske and Subbarow (1925).

TABLE III

*Observations pertaining to the Animals and Results with regard to the Humeri*†

| Particulars | Protein content of diet | | | | | |
|---|---|---|---|---|---|---|
| | 5% | 10% | 12·5% | 15% | 17·5% | 20% |
| Initial Body Weight (g.) | 258 | 261 | 254 | 261 | 260 | 261 |
| Final Body Weight (g.) | 246 | 283 | 274 | 312 | 312 | 312 |
| Daily food Intake (g.) | 11·80 | 11·56 | 12·72 | 12·60 | 12·77 | 12·38 |
| Dry, Fat-free weight of Humeri (g.) .. | 0·209 | 0·221 | 0·225 | 0·235 | 0·230 | 0·232 |
| Ash weight of Humeri (g.) .. .. | 0·140 | 0·149 | 0·149 | 0·158 | 0·156 | 0·157 |
| Calculated Ash content (%) .. .. | 67·07 | 67·85 | 68·18 | 68·80 | 68·21 | 68·34 |
| Length of Humeri (mm.) .. .. | 25·15 | 26·49 | 27·39 | 28·32 | 28·14 | 28·21 |
| Ca/P Ratio of Ash: | 0·029 / 0·012 | 0·031 / 0·013 | 0·032 / 0·013 | 0·034 / 0·014 | 0·033 / 0·014 | 0·036 / 0·014 |

† The data are shown as averages.

## RESULTS AND DISCUSSION

In Table III are given the length, weight and ash content of the humeri of the rats at 5, 10, 12·5, 15, 17·5 and 20% protein intake respectively. All the data related to the humeri are the means of the average obtained with the two bones from the individual animal in each group. It is seen from Table III that the gain in weight of the animals varied in the same manner as the protein content of the food. The animals receiving 20% protein gained on an average 5 gm. while those receiving 5% protein lost on an average 16 gm. The humeri of the groups receiving 5 and 10% protein diet suffered a significant reduction in weight of the fat-free dry bone and

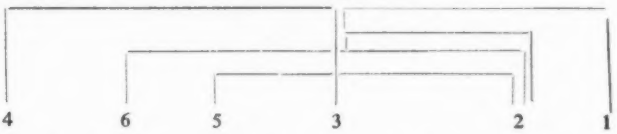
its ash weight. Increasing the protein from 12·5 to 20% in the diet effected a gradual increase in the skeletal growth and also in the weight of the ash. Maximum skeletal growth and ash weight were found at 15% protein level. Further with an increase in the prawn protein in the diet from 10 to 12·5% there is an increase in the skeletal growth as well as the ash content. It is seen that 12·5% protein level is the minimum level for normal skeletal growth. The 5 and 10% protein diets are inadequate to support normal skeletal constitution in mature rats equal to that of a diet containing 15% prawn protein.

TABLE IV

| | Variations due to | | | |
|---|---|---|---|---|
| | Groups | Treatment | Error | Total |
| Sum of Squares .. | 4·7102013 | 78·8669453 | 21·1676687 | 104·7528183 |
| Degrees of freedom | 9 | 5 | 45 | 59 |
| Variance of Mean square .. | 0·524245 | 15·77338966 | 0·47039264 | .. |

$F = 33{\cdot}53239043$. $1\% = 3{\cdot}45$ $t_{45}$ at 1% value $= 2{\cdot}69$.

Difference of two means greater than 0·82508218 will be significant.




Statistical data obtained for these results confirmed the superiority of 15% prawn protein diet over the other diets, with regard to skeletal growth and body weight. The optimum level protein for normal skeletal growth was found to be 12·5% prawn protein.

The effect on body weight produced by feeding the mature rats for 30 days with diets containing 5, 10, 12·5, 15, 17·5 and 20% prawn protein was also determined. It is seen from Table III that 5% prawn protein did not support the body weight, of mature rats while diets containing 10, 12·5, 15, 17·5 and 20% permitted an increase in body weight in approximate proportion to the protein contents.

The composition of the bone ash in all the groups was not affected by protein deficient diets, as can be seen from the Ca: P ratio in Table III.

## SUMMARY AND CONCLUSION

The body weight and humeri of mature rats fed for 30 days with diets containing 5, 10, 12·5, 15, 17·5 and 20% prawn protein have been determined. The following conclusions are drawn:

1. A diet containing 5% prawn protein will not support the body weight of mature rats, while diets containing 10, 12·5, 15, 17·5 and 20% protein permit an increase in body weight in approximate proportions.

2. A diet containing 12·5% prawn protein supports a degree of skeletal constitution in mature rats equal to that of a diet containing 15% prawn protein.

3. 5 and 10% prawn protein diets are inadequate to support normal skeletal constitution.

4. The compositions of the bone ash is not affected by feeding prawn protein deficient diets to mature rats.

## LITERATURE CITED

1. Armstrong, W. O. .. *Pro. Soc. Exp. Biol. Med.*, 1946, **61**, 358.
2. Clark, E. P. and Collip, J. B. .. *J. Biol. Chem.*, 1925, **63**, 461.
3. Conner, R. T. and Sherman, A. C. .. *Ibid.*, 1936, **115**, 695.
4. Fiske, C. H. and Subbarow, Y. .. *Ibid.*, 1925, **66**, 375.
5. Osborne, J. B. and Mendel, L. B. .. *Ibid.*, 1926, **69**, 661.
6. Quinn, E. S., King, C. G. and Dinut, B. H. *J. Nutrition*, 1929, **2**, 7.
7. Sherman, H. C. and Booher, L. E. .. *J. Biol. Chem.*, 1931, **93**, 93.
8. Sherman, C. S., Campbell, H. L. and Sherman, H. C. .. *Ibid.*, 1941, **137**, 627.
9. ———— and Sherman H. C. .. *Ibid.*, 1938, **126**, 381.
10. Van Duyne, F. O., Lanford, C. S. Toepfer, E. W. and Sherman, H. C. *J. Nutrition*, 1941, **21**, 221.

# PRELIMINARY STUDIES ON CERTAIN CHANGES IN THE PLANKTON AND HYDROLOGICAL CONDITIONS ASSOCIATED WITH THE SWARMING OF *NOCTILUCA**

By R. Raghu Prasad and R. Jayaraman

(*Central Marine Fisheries Research Station, Mandapam Camp*)

Received November 14, 1953

(Communicated by Dr. N. K. Panikkar, F.A.SC.)

An effect of swarming of *Noctiluca* on the 'choodai' fishery was reported by one of us (Prasad, 1953) and there it was mentioned that the appearance of these swarms was accompanied by certain changes in the chemical and biological environment. There are several instances of antagonistic or lethal reactions on zooplankton caused by the presence of swarms of dinoflagellates and some of the well known examples of this phenomenon have been given by Sproston (1949). Lucas (1938) termed such interrelations the 'non-predatory' relationship and postulated the theory that these antagonistic or associative interrelations and ecological succession in plankton and elsewhere are the effects of metabolic by-products or external metabolites which he called *ectocrines*. Similarly, the sudden appearance in large numbers of any one member of the complex marine community of animals and plants may be accompanied by pronounced changes in the hydrological conditions of the environment. These changes in most cases are of a temporary nature lasting only for the period of appearance of the swarms.

Swarms of *Noctiluca* were observed in the inshore waters of the Palk Bay and the Gulf of Manaar in 1952. These swarms appeared spasmodically in varying intensities from March to November and were more often observed in the Palk Bay than in the Gulf of Manaar. During this period there were also days when these dinoflagellates were not present at all in this area.

The material consists of routine collections of plankton and surface water from inshore stations in the Palk Bay and the Gulf of Manaar made during March to November 1952. In the enumeration of diatoms, *Noctiluca* and copepods the procedure adopted by Prasad *et al.* (1952) has been followed. The methods of collection and analyses of water samples are the same as described in an earlier paper by one of us (Jayaraman, 1951).

* Published with the permission of the Chief Research Officer, Central Marine Fisheries Research Station, Mandapam Camp, South India.

The present paper gives a preliminary account of the changes in the nature of plankton and attempts to correlate certain hydrological factors with the appearance of swarms of *Noctiluca* in the neighbourhood of Mandapam. Although the data collected were not concerned directly with an investigation into the causes of such a swarming, the interest stimulated by the association of the various hydrological and biological factors observed justify the purpose of this brief report.

Several instances of dinoflagellate swarms have been reported in the literature and a more or less complete review of the subject on " Blooming in the sea " has been given by Galtsoff (1948). But detailed investigations into the nature of the chemical changes associated with such phenomena are rather meagre. The only observation of importance is by Ketchum and Keen (1948) who have reported unusual phosphorus concentration in the Florida ' red tide ' sea water. During the ' red tide ', believed to have been caused by the appearance of dense populations of the dinoflagellate *Gymnodinium*, these authors have found the total phosphorus content in the surface waters to be two and a half to ten times the maximum to be expected in the sea. The occurrence of such high phosphorus concentration was considered to be the direct effect of the swarming of *Gymnodinium*.

Among the various hydrological factors examined in the present study during the swarming of *Noctiluca*, certain interesting correlations between salinity and silicates and also the occurrence of silicates in very high concentration—15 to 25 $\mu$g.—at. Si/L which is three to five times the usual concentration of silicates in local waters—were observed. Unlike Ketchum and Keen (1948) no unusual phosphorus concentrations were noticed by the present authors. Figures 1 and 2 show the distribution of salinity and silicates in the Palk Bay and the Gulf of Manaar respectively during this period, March to November 1952. It may be seen from these figures that high silica content and high salinity were occurring almost together. The months during which salinity was high coincide with the season of the south-west monsoon and from the charts for current systems in the Bay of Bengal compiled by Sewell (1929) it could be seen that in these months the local waters are influenced to a large extent by the more saline waters of the Indian Ocean and the Arabian Sea. Waters of the 'open ocean' generally contain very small quantity of silicates in solution. River and estuarine waters on the other hand are relatively richer in dissolved silicates (Harvey, 1928). Large concentration of silicates in an area is, therefore, usually associated with lowering in salinity caused by admixture with river water. The combination of high salinity and high silica content, as has been observed in the present instance is of unusual

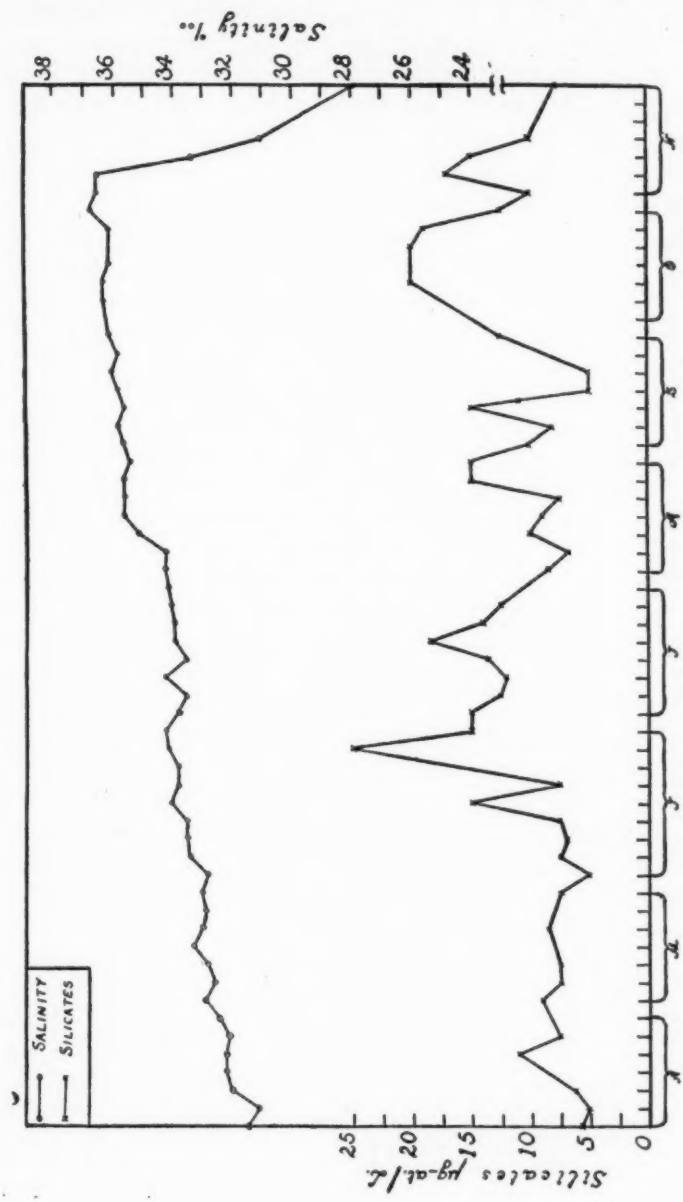



FIG. 1. The distribution of salinity and silicates in the Palk Bay during April to November 1952.

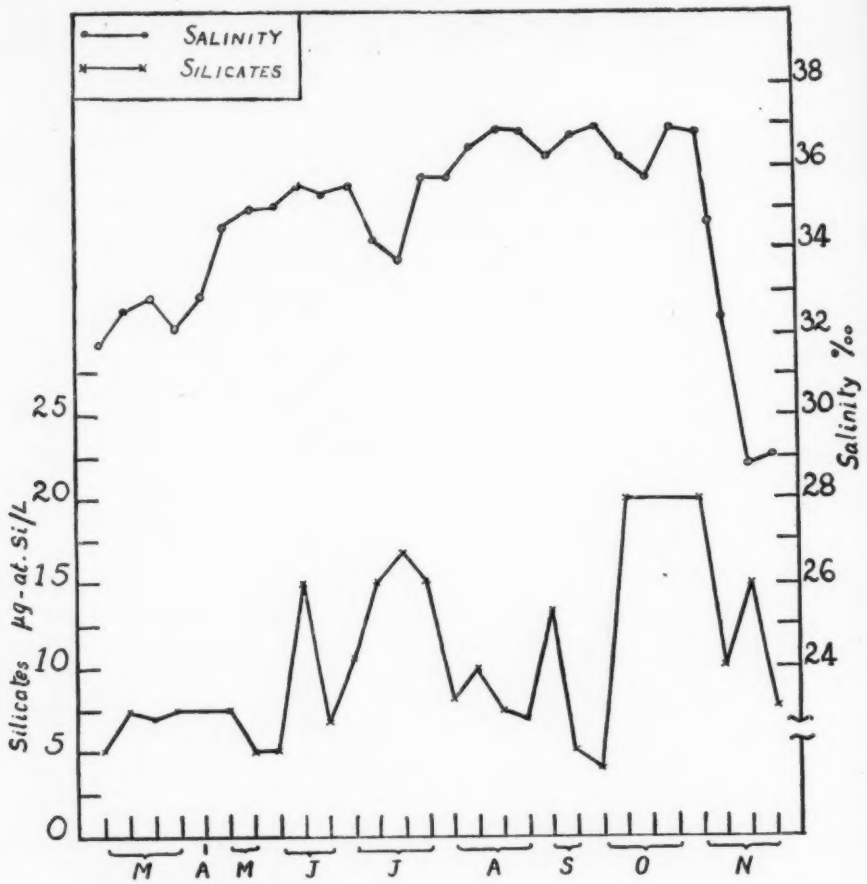



FIG. 2. The distribution of salinity and silicates in the Gulf of Manaar during March to November 1952.

occurrence in this area and may, therefore, be considered as one of the important hydrological changes during this period of *Noctiluca* swarming. The probable explanation for the occurrence of silicates in such high concentration in the surface waters is that there must have been active regeneration *in situ* of silicates from the siliceous frustules of the diatoms. The process of the solution of silica skeletons in the sea water is, however, not so simple as has hitherto been believed to be. Cooper (1952) in an interesting paper on the distribution of silicates in the North Atlantic has mentioned that the intact skeletons of diatoms are not easily acted upon by sea water,

There should be some mechanism by which freshly fractured surface of silica are brought into contact with water before solution could take place. The commonest way of "exposing fresh silica surfaces by fracturing will occur in the guts of the herbivores which grind or triturate their food". Such a process of "comminution" will facilitate considerably the solution of silica in sea water. A change similar to this process of "comminution" of diatom skeletons might be taking place during the active feeding of diatoms by the *Noctiluca*. It is therefore, possible that when there is swarming of *Noctiluca* more and more of the covering of the diatom cells are brought into solution thereby contributing to the increase in the silica content of the surface waters.

These changes in the hydrological conditions and the presence of *Noctiluca* in very large numbers are bound to have direct effects on some of the members of the plankton community. Johnstone, Scott and Chadwick (1924) wrote: "Also we are pretty sure that the plankton communities influence each other—that there are what we may call group symbiosis on the great scale so that the kind of plankton which we may expect to be present in a certain sea-area must depend to some extent, on the kind of plankton which was previously present." In recent years a closer study of the biodynamics has brought out the great intricacy of the net-work of biological inter-relationships and more and more evidence has been accumulating regarding a type of organic relationship which may broadly be differentiated from those interrelationships which concern with feeding in the widest sense. These non-predatory relationships relate to the external metabolites which are ecologically potent and bear important significance for the organisms producing them as well as for the other members of the community. Metabolites harmful to some may be beneficial to others and the effects of these metabolites in the ecological succession cannot be over-emphasised. For a review on the subject reference may be made to Lucas (1947).

The most significant feature observed in the plankton on the days when swarms of *Noctiluca* were present was the appreciable decrease in the number of copepods in the areas where the swarms occurred. There was also a decline in the number of some of the other zooplankters although not of the same magnitude as that of copepods. This phenomenon of inverse relationship was characteristic throughout the season both in the Gulf of Manaar and the Palk Bay (Figs. 3 and 4) and is undoubtedly an example of 'animal exclusion' due to the effect of a potent ectocrine of *Noctiluca*, the nature of which is yet unknown. Another interesting phenomenon noticed during the same period was the succession of varying intensities of diatom peaks following those of the *Noctiluca*. This is more evident in the Palk Bay

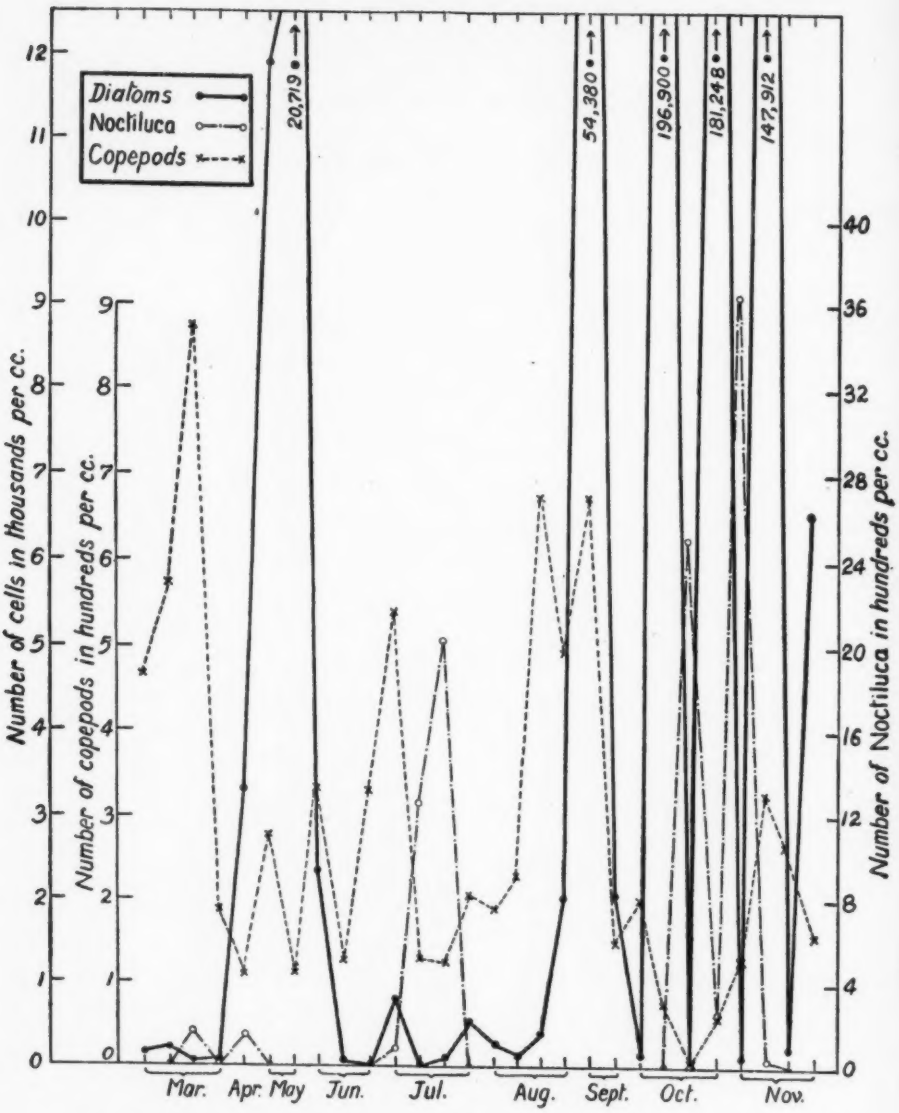



FIG. 3. Showing the distribution of *Noctiluca* copepods and diatoms in the Gulf of Manaar during March to November 1952.

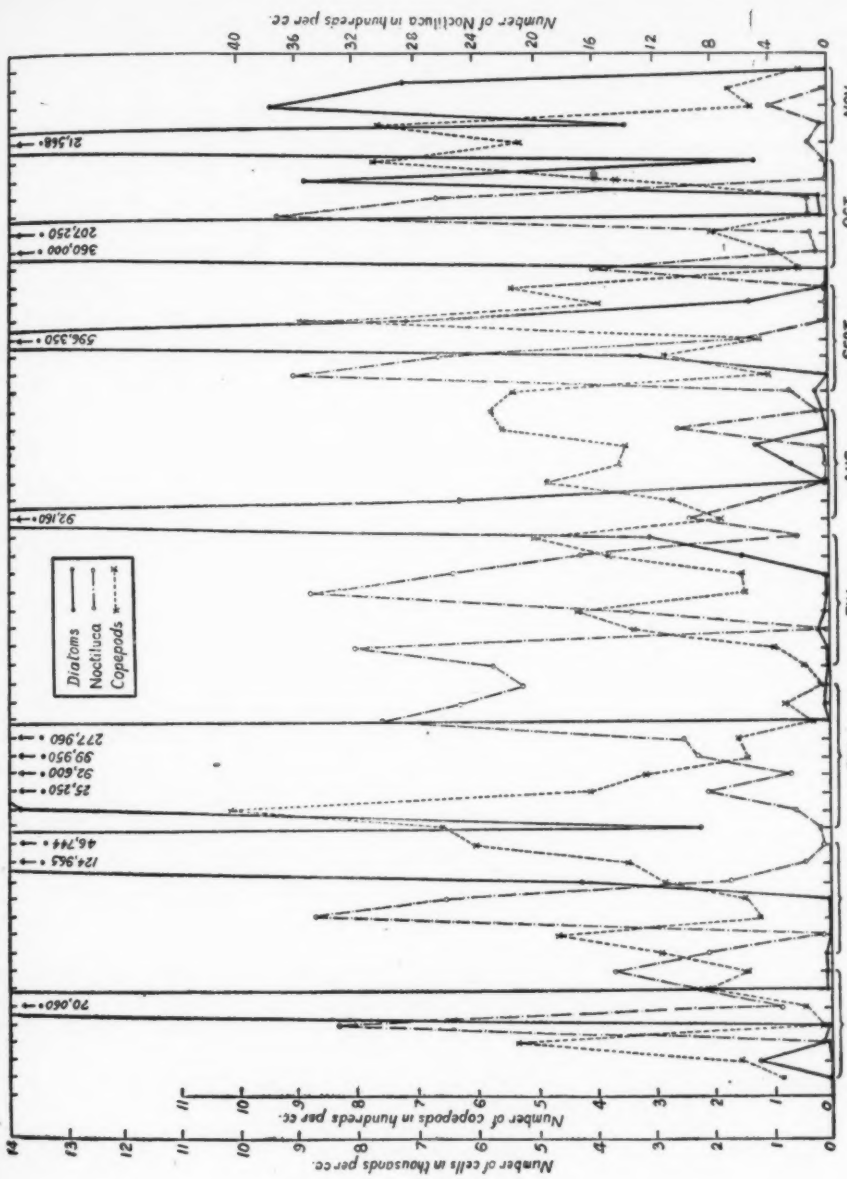



Fig. 4. The distribution of *Noctiluca* copepods and diatoms in the Palk Bay during April to November 1952.

where there were greater concentrations of *Noctiluca* populations. It was mentioned earlier that the *Noctiluca* swarms were accompanied by an appreciable increase in the silicate content of the surface water. There were also indications that the phosphate and nitrate showed a slight increase immediately following most of the *Noctiluca* peaks. Therefore, it is possible that the high silicate content together with an increase in the other two important nutrient salts created conditions favourable for the rapid multiplication of diatoms resulting in distinct peaks. It will be further noticed that when *Noctiluca* was present in great numbers the diatom level was exceedingly low which may be the effect of active feeding on diatoms by these dinoflagellates. The increase in the silicate content of the sea water consequent on the feeding by the *Noctiluca* on the diatoms and thereby facilitating quicker solution of silicates, as already pointed out earlier in this report, support the assumption that the significant reduction in the diatoms when there were large *Noctiluca* populations was due to consumption. Thus, there appears to be two distinct types of relationships functioning at the time of *Noctiluca* swarms in this area—one concerned with the feeding and the other the non-predatory relationship in which the *ectocrines* function as the mediating substance. A regular ecological succession in the occurrence of *Noctiluca*, diatoms and copepods and also a decimation of zooplankton in general and copepods in particular were observed during the swarming of *Noctiluca*.

The authors wish to thank Dr. N. Kesava Panikkar for his critical comments.

## REFERENCES


Cooper, L. H. N. .. "Factors affecting the distribution of silicate in the North Atlantic Ocean and the formation of N. Atlantic Deep water," *Jour. Mar. Biol. Assoc.*, 1952, **30**, 511–26.

Galtsoff, P. S. .. "Red tide: Progress report on the investigation of the cause of the mortality of fish along the west coast of Florida conducted by the U.S. Fish and Wildlife Service and co-operating organizations," *U.S. Fish and Wildlife Service Special Scientific Rept.*, 1948, No. 46, 1–44.

Harvey, H. W. .. *Biological Chemistry and Physics of Sea Water*, Cambridge Uni. Press, 1928, 1–194.

Jayaraman, R. .. "Observations on the chemistry of the waters of the Bay of Bengal off Madras City during 1948–49," *Proc. Ind. Acad. Sci.*, 1951, **33**, Sec. B: 92–99.

Johnstone, J., Scott, A. and Chadwick, H. C. .. *The Marine Plankton* (A handbook for students and amateur workers). The University Press, Liverpool, 1924, 1–194.

Ketchum, B. H. and Jeen Keen "Unusual phosphorus concentration in the Florida 'Red tide' sea water," *J. Mar. Res.*, 1948, **7**, 17–21.

Lucas, C. E. .. "Some aspects of integration in plankton communities," *Joun. Cons. Per. Int. l'Expl. de la Mer*, 1938, **13**, 309–22.

———— .. "The ecological effects of external metabolites," *Biol. Reviews*, 1947, **22**, 270–97.

Prasad, R. R. .. "Swarming of *Noctiluca* in the Palk Bay and its effect on the 'choodai' fishery, with a note on the possible use of *Noctiluca* as an indicator species," *Proc. Ind. Acad. Sci.*, 1953, **38**, Sec. B: 40-47.

——, Bapat, S. V. and Tampi, P. R. S. "Observations on the distribution of plankton at six inshore stations in the Gulf of Manaar," *J. Zool. Soc.*, India, 1952, **4**, 141–151.

Sewell, R. B. S. .. "Geographic and oceanographic research in Indian waters—Pt. V. Temperature and salinity of the surface waters of the Bay of Bengal and Andaman Sea, with references to the Laccadive Sea," *Mem. Asiatic Soc.*, Bengal, 1929, **9**, 207-356.

Sproston, N. G. .. "A preliminary survey of the plankton of the Chu-San region, with a review of the relevant literature," *Sinensia*, 1949, **20**, 58–161.

## ADDENDUM

Since this paper was sent to the press, Subrahmanyan (*Proc. Ind. Acad. Sci.*, 1953, **39**, Section B, 118–127) also has recorded high silicate values during the occurrence of *Noctiluca* peaks.

# THE PROBLEM OF THE APPARENT DISCONTINUOUS DISTRIBUTION OF *HARPODON NEHEREUS* (HAMILTON)

By B. Sundara Raj, M.A., Ph.D. (Liverpool), F.N.I., F.Z.S.I.
(*Department of Fisheries, Colombo 3, Ceylon*)

Received October 5, 1953
(Communicated by Prof. B. R. Seshachar, F.A.SC.)

*Harpodon nehereus* popularly called Bummaloes or Bombil and best known as ' Bombay Duck ' (when dried) has a wide distribution along the coasts of East Africa, Asia, Indonesia and China. In Africa it is known only from Zanzibar. In India it has been recorded hitherto from the Gulf of Cambay to Ratnagiri on the west coast,* and from Madras to Chitagong on the east coast. In Burma it occurs along the Arakkan Coast and abundantly in the estuaries of the Irawaddy. In Malaya it is reported from Penang, the Straits of Malacca and Singapore. In Indonesia it is known from Sumatra, Java and Borneo. In China it has been taken at Canton, Swatow, Amoy, Ningpo, Chusan, Woosung and Newchwang.†

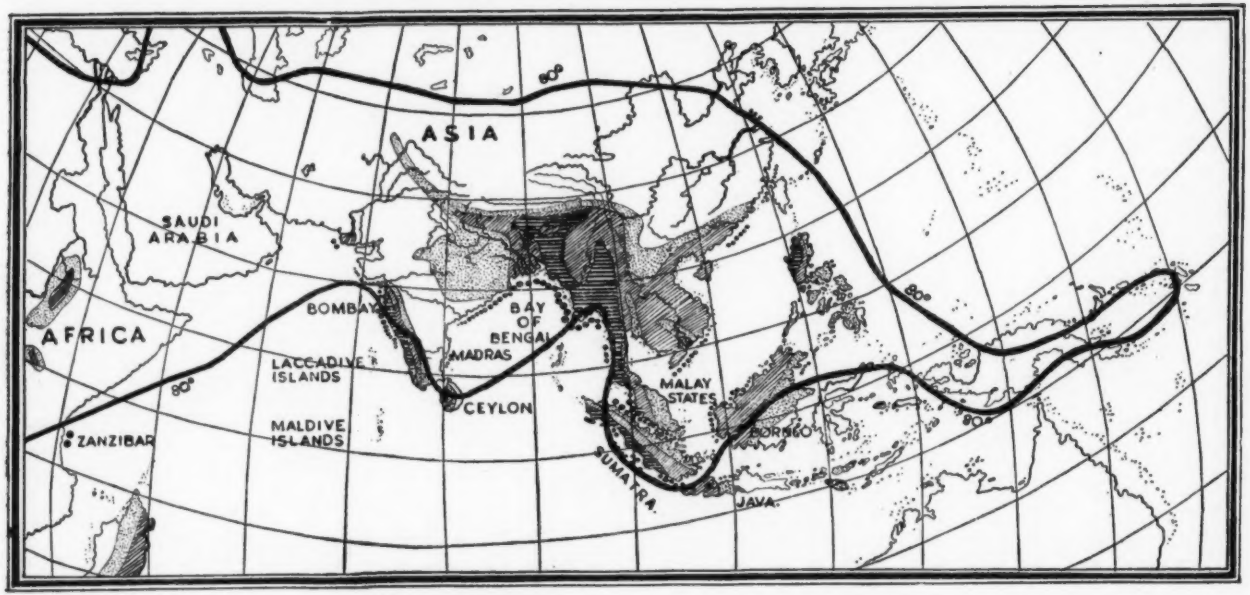
My recent enquiries have helped to fill some gaps in the hitherto known range of the fish. I have ascertained from Dr. M. R. Quereshi, Director of Fisheries, Sind, that the fish is caught in Karachi but not in commercial quantities. Dr. Setna, Director of Fisheries, Bombay, states that the fish does not occur in the Gulf of Cutch but is abundant in the Gulf of Cambay. On the east coast the author has found the fish on very rare occasions in the Cauvery delta, close to Point Calimere. Mr. J. S. Scott of the Fisheries Department, Federation of Malaya and Singapore, informs me that the fish is found along the west coast of Malaya, but it is abundant only in the Central Sector of the west coast in Selangor area and that the fish is absent on the east coast. In Sumatra, the fish has been recorded from the west coast but is abundant on the east coast. Mr. Scott says it occurs " in the regions of Ketaman, Indragiri and Karimoen." Dr. Hardenberg informs me that ' Bombay Duck ' has not been recorded from the south coast of

* In the 'Fishes of Malabar', page 202, Day states "This fish which is so extensively numerous in North Malabar and Bombay rarely if ever extends its range so far south as Cochin". There is no recent record of the fish south of Ratnagiri.

† I am indebted to Dr. K. S. Misra of the Zoological Survey of India for this information.

## MAP OF SOUTH EAST ASIA

(SHOWING OCCURRENCE OF THE BOMBAY DUCK)



Java: though an extensive search was made by him, only stray single specimens are taken on the north coast of Java. The Chief Secretary to the Government of Sarawak says that large quantities of the fish are frequently caught between September and November on the west coast of Borneo. The fish is not known from Labuan to Sandakan along the north-west coast of British North Borneo. Mr. J. A. Tubb, Director of Fisheries, found in August 1947 and 1948 on the east coast a few specimens at Tawau fish market. The Director-General of Fisheries, Thailand, says it is not known to occur in Thailand. The Director of the Oceanographical Institute, Cauda, Indo-China, informs me that the fish appears in commercial quantities on the Saigon coast. The Director of Fisheries, Philippine Islands, as well as Dr. Herre assure me that ' Bombay Duck ' does not occur in the Philippines. The Fisheries Officer, Hongkong, reports that the fish is found all round the year at the mouth of the Pearl river near Macau, the peak season being July to September.

Chart shows the occurrence and relative abundance of the fish so far ascertained throughout its range. The extreme limits of its distribution are roughly 40° North and 10° South latitude and 40° to 122° East longitude.

A close study of the geographical and oceanographical features of this region reveals the following remarkable co-relations between them and the distribution of the fish.

(i) 80° *F. July Isotherm.*—The warm side of the summer (July) 80° F. isotherm reduced to sea-level and the limits of distribution of the ' Bombay Duck ', except for Java in the extreme south and Newchwang in the extreme north, almost exactly coincide (*vide* Chart). It is about July that the fish appear in great shoals everywhere. In Africa this isotherm passes close to Zanzibar where the fish occurs. On the west coast of India, the isotherm excludes the section below Bombay to Cape Comorin as well as the west coast of Ceylon, where the fish is rare or absent. It runs just inland along the Tenasserim coast of Burma, where the fish is little known, but includes the Central Sector of the west coast of Malaya and the east and west coasts of Sumatra where the fish is well known. It excludes Java where stray specimens occur on the north coast closest to the isotherm. The east and west coasts of Borneo and the Pacific coast of Indo-China and China to somewhat north of the 40° N. parallel, which is the northernmost limit of distribution, all lie within the 80° F. summer isotherm for July. The known limits of distribution of the fish fit so exactly with this isotherm that it is not possible to dismiss it as a mere coincidence.

In the Far East, the 80° F. July isotherm includes New Guinea where curiously enough another allied species *Harpodon novæguinensis* sp. nov. occurs which Dr. Hardenberg has recently described. According to him, the new species lives in exactly the same conditions as *Harpodon nehereus.*

The climate on land is determined by temperature and precipitation (rain, dew, etc.), while in the sea, the factors are temperature and salinity. It is well known that the climate of coastal seas closely corresponds to that of the adjacent land. There is also a direct relationship between the air temperature at sea-level and that of the surface sea-water. The isotherm of the atmosphere may generally be assumed to represent the temperature of the surface water with a margin of 1 or 2 degrees either way. The maximum summer temperature of the surface sea-water in Indian seas is about 84° F. reached in April or May. With the onset of the south-west monsoon, the surface water cools and is about 82° F. in July.

If the above co-relation between the 80° F. isotherm and the distribution of the fish is accepted, it is logical to conclude that the fish is very sensitive to temperature conditions and that the atmospheric temperature of 80° F. at sea-level with its closely related surface sea-water temperature is evidently the optimum for the mass migration of the ' Bombay Duck '.

(ii) *The South-west Monsoon.*—There is a close co-relation between the south-west monsoon and the ' Bombay Duck ' fisheries both in regard to time and place. Chart shows the rainfall during the south-west monsoon and the occurrence and relative abundance of the fish throughout its extensive range. Though ' Bombay Duck ' occurs all the year round in many places, the season for its greatest abundance is from May or June to October, which coincides with the south-west monsoon. After the monsoon, fishing may continue for varying periods and intensity up to December or later.

For instance, Dr. Setna says that the abundant season in Bombay and vicinity is from June to September. Messrs. Bapat, Banerji and Bal state that the fishing season on the Konkan and Gujarat coasts starts sometime in September just after the south-west monsoon and continues for about 4 or 5 months. They, however, agree with Dr. Setna that the season starts much earlier, for they say that during the monsoon the fishing activities are confined to inshore waters. According to Pillai, in the Mutlah river, West Bengal, the fish are caught from about April to December. According to Mr. J. S. Scott, the fish abound in the Central Sector of the west coast of Malaya during " the middle part of each year". So far as he knows the fish are very little or not known at all on the east coast. In British North Borneo a few fish have been seen in August 1947 and 1948 on the east coast

at the Tawau fish market. On the west coast of Borneo in Sarawak quite large quantities of fish are frequently caught from September to November. On the China coast the peak season is from July to September. Thus the shoaling season for 'Bombay Duck' throughout its wide range starts during the south-west monsoon.

Moreover, the places of abundance of the fish are along stretches of the coast directly or indirectly affected by the south-west monsoon. The fish is rare in Zanzibar and Karachi, which are only partially affected by the south-west monsoon. It is abundant on the west coast of India which directly intercepts the monsoon. On the east coast the fish are rare on the Coramandel coast. Though the Cauvery receives the south-west monsoon floods, the coast itself is not touched by the monsoon. The catches increase in the northern Circars, Orissa, West Bengal, East Pakistan and Burma, which receive the full force of the south-west monsoon. The fish abounds on the west coast of Malaya, it is not so abundant on the east coast of Sumatra, but is plentiful on the west coast of Borneo, the east coast of Indo-China and China, all of which receive heavy south-west monsoon rains. There is thus an unmistakable co-relation between the south-west monsoon and the 'Bombay Duck' fisheries.

Chart however, shows some apparent anomalies which call for an explanation. The 'Bombay Duck' is conspicuous by its absence from the Gulf of Cutch, the west coast of India below Ratnagiri and from the west coast of Ceylon, Thailand and the Philippines, though all these areas are subject to heavy south-west monsoon rains. A close scrutiny of the map, however, suggests that two more geographical features affect the occurrence and abundance of the fish. These are the estuaries of large rivers and islands which skirt the coast. If these are taken into account the unexplained abundance and absence of the fish in some sections of the coast can be accounted for.

(iii) *Estuaries and Islands.*—Many writers have observed that 'Bombay Duck' congregate in estuaries, but none so far has suggested a co-relation between islands and the absence of the fish from the adjacent coast. The Gulf of Cutch and the Gulf of Cambay intercept the south-west monsoon, but the former receives no large rivers to attract the fish. The chief west flowing rivers of Peninsular India, the Tapti and the Nurbada, both empty into the Gulf of Cambay. It is to the south-west monsoon floods in these rivers that the abundance of the fish in the Gulf of Cambay and along the coast down to Bombay should be attributed.

From Ratnagiri to Cape Comorin and on the west coast of Ceylon, though the south-west monsoon is very heavy, the absence of large rivers, the presence of a chain of banks and islands effectively screening the coast and the fact that this area is outside the 80° F. summer isotherm would seem to account for the total absence of the fish.‡

On the east coast the occurrence of the ' Bombay Duck ' in the Cauvery Delta seems to be due to the south-west monsoon floods brought down by the Cauvery, though the Coramandel coast itself is not touched by the monsoon. Further north the estuaries of the Kistna, Godavari, Mahanadi, Ganges, Brahmaputra and the Irawaddy are all noted for the great abundance of ' Bombay Duck'. The fish are rare or absent along the South Tenasserim coast§ and along the west coast of Siam as this coast is sheltered by the Moscos Islands, the Mergui Archipelago and other islands. They abound in the Central Sector of the west coast of Malaya as the monsoon directly strikes the coast.

In Sumatra, though it is the west coast that intercepts the south-west monsoon, the fish are more abundant on the east coast, evidently because the entire drainage of this island is towards the east into the Straits of Malacca and also there is a chain of islands along the west coast. This probably accounts for the somewhat curious abundance of the fish in the Straits of Malacca.

The fish are absent on the east coast of Malaya and in the Gulf of Siam as these are sheltered from the monsoon by the Malay Peninsula and have no rivers of any importance. The fish reappear in large numbers in the estuary of the Mekong in Indo-China which is the chief river of this area. The ' Bombay Duck ' are unknown in the Philippines as there are no large rivers or estuaries to attract them. Also most of these islands are outside the 122° E. longitude which seems to be the extreme limit of distribution.

---

‡ Dr. Hora (1934), p. 649 attributes the scarcity of the fish from Bombay to Madras to the absence of: (i) large rivers, (ii) locally heavy rain.

Actually the rainfall during the south-west monsoon is far heavier on the Malabar and Canara coasts than in Bombay. For June and July the normal rainfall at Mangalore is 76" and Calicut 65·5 against 43·3 at Bombay. Yet the fish are totally absent. He was also not aware in 1934 of the occurrence of the fish on the Coramandel coast, south of Madras. Further, though there are no large rivers at Penang or Malacca, the fish are extensively caught there.

§ Col. Sewell informs me that young 'Bombay Duck' were taken by Investigator in tow nets along the south Tenasserim coast. The larger specimens were found on the seaward face of the islands of the Mergui Archipelago.

The co-relations discussed above revive interest in Dr. Gunther's belief that *Harpodon nehereus* is evidently an inhabitant of considerable depth and periodically comes nearer the surface. Gunther believed that *Harpodon nehereus* belongs to the deep sea on account of its remarkably close resemblance in form and colour to deep sea fish. Its phosphorescent and semi-translucent milky white colour, gelatinous consistency, large mouth with loosely articulated lower jaw armed with recurved teeth, are all characteristics which are strongly reminiscent of deep sea species. Recent researches and my own studies seem to provide additional grounds for this view.

(i) All the other known species of Harpodon except *H. novæguinensis* are deep sea forms.

| | |
|---|---|
| *Harpodon microchir* .. | .. Gunther, Japan, depth not known. |
| *Harpodon squamosus* .. | .. Alcock, Indian Ocean, 120–300 fathoms. |
| *Harpodon mortinseni* .. | .. Hardenberg, Bali Sea, 300 m. |

(ii) The predominant fish in the stomach contents of examples from Bombay according to Dr. Hora was *Bregmaceros mcClellandi*, Thompson, which is widely distributed through the Indo-Pacific from the surface to a possible depth of 2,000 m. Norman (1950)¶. Messrs. Bapat, Banerji and Bal, however, after examining the stomach contents of 217 specimens, found *B. mcClellandi* to be of less importance than young *H. nehereus*. The examination of their Table II shows that Bregmaceros is quite important in some specimens examined by them. It is therefore not improbable that Bregmaceros is predominant only in fresh arrivals on the Bombay coast. After their arrival on the coast young Harpodon which abound in the shallow inshore water may replace *B. mcClellandi* as the chief fish consumed. This observation needs further investigation for its confirmation. Bregmaceros was not found by Pillai in the contents of 700 specimens from the River Matlah (West Bengal) nor by Dr. Hardenberg in samples from the east coast of Sumatra apparently because *B. mcClellandi* frequents only the open sea.

(iii) In Bombay where the 'Bombay Duck' fishery has been closely studied, Setna, Bapat, Banerji and Bal agree that the fish mostly swarm in the shallow inshore waters during the south-west monsoon rains. Later in September and October they are taken in stake nets 4 or 5 miles from the shore. Still later, they are caught from places 10 to 12 miles away from the shore in the open sea. This seems to indicate an onshore migration

¶ The fish were caught in open tow nets hauled from a depth of 2,000 m. There is thus no certainty that the fish were actually caught at 2,000 m.

during the south-west monsoon and an off-shore movement into deeper water of the open sea after the monsoon. A similar trend is noticeable also in the catches made by the trawler, " Golden Crown " in the Sunderbans and off the Orissa coast.

| | |
|---|---|
| 1st– 5th October | .. 450 lb. |
| 11th–15th October | .. 350 lb. |
| 12th–29th October | .. 315 lb. |
| 24th Nov.–3rd Dec. | only 100 lb. |

This progressive diminution of the catch from October to December as the ' Bombay Duck ' season is nearing its end, is significant and indicates that the fish are leaving the coast and receding into deeper water of the open sea.

(iv) Lastly Setna found only undeveloped gonads in catches during the peak of the fishing season (June to October) whereas the fish taken far out at sea in February and March contained ripe eggs and active spermatozoa. Messrs. Bapat, Banerji and Bal confirm this observation. They state that " The absence of eggs and larval forms in inshore waters and as well as on some of the actual fishing grounds indicate that the spawning takes place somewhere beyond this region. This view is further supported by the fact that a dozen specimens were taken in a trawl net about 50 to 60 miles north-west of Bombay light ship in November 1948. On examination they were found to be all females possessing ripe or ripening ovaries." This is sufficient indication that there is a definite onshore and off-shore movement of shoals at the commencement and after the close of the south-west monsoon.

The obvious explanation stressed by Dr. Hora that lowering the salinity by the monsoon rains induces the landward migration, is not adequate to account for the mass movement of the fish only in June and July. The north-east monsoon in November and December is also heavy on some sections of the coast particularly in Malaya, Sumatra and Borneo frequented by the fish and yet there is no indication of another mass movement of the fish towards the coast in that season. On the other hand the fish have been actually observed to leave the coast both in Bombay and Bengal. The temperature conditions as indicated by the significant co-relation between the ' Bombay Duck ' fisheries and 80° F. summer isotherm, therefore, appear to be more important than salinity. The combination of both low salinity and higher temperature is apparently the optimum required by the fish to start their landward journey. Once the fish arrive on the coast their return to the open sea seems to be gradual, some immature fish lingering in estuaries all the year round.

On the above premise the true significance of the co-relation between islands and the absence of the fish on the coast close-by becomes apparent. As the fish approach the shore in response to the stimulus provided by the monsoon floods and the summer temperature, they are obstructed by the islands which may divert them to open sections of the coast. The distribution of the fish on the west coast of India and the Malay Peninsula provides the best examples.

The chief objection, however, to considering *Harpodon nehereus* as a deep sea fish, is that it has never been taken from any great depth. With the exception of the Dana expedition no systematic attempt to collect fish from intermediate levels of water in the deep sea has been made. While a great deal is known regarding fish inhabiting the bottom or the surface of the sea, very little is yet known about bathypelagic or mesopelagic species, which inhabit intermediate levels of the deep sea. All the evidence at my disposal points to the ' Bombay Ducks ' being a bathypelagic or mesopelagic species. The ' Bombay Duck ' and the allied *H. novæguinensis* are evidently derived from a true deep sea stock but have adapted themselves to life in intermediate levels.

As the submarine banks arising from depths of 1,000 fathoms or more (for instance, the Luccadives and Maldives) seem to act as effective barriers to the shoreward migration of the fish, and as depths of the sea upto 2,000 fathoms adjoin these islands and the coasts frequented by shoaling ' Bombay Duck ' it is not improbable that the home waters of these fish are in the open sea of about 2,000 fathoms depth.

Dr. de Beaufort in a letter to me has suggested that lower salinity and high temperature, though they apparently attract the fish, cannot be the primary reason for the migration. He says that since spawning has been ruled out, feeding may prove to be the reason for the mass movement of the fish to shallow water.

Dr. de Beaufort's theory is certainly true of young fish. Analysis of the food of the fish has been made by Sewell, Hora, Bapat, Banerji and Bal and Pillai. According to Colonel Sewell the young are plankton feeders. I have already stressed the evidence afforded by *Bregmaceros mcClellandi* in the food of the adult. The other fish and crustaceans consumed are all common species found on the coast. They seem to furnish no further clue, beyond their relative abundance in coastal waters, to the migration of the fish.

Could not spawning be the purpose of the migration after all? The data available seem to me to indicate that the ' Bombay Duck ' approaches

the warm and less saline coastal waters primarily for breeding. Messrs. Bapat, Banerji and Bal found fish with ripe or maturing gonads far out at sea. No eggs or larvæ have so far been found in coastal waters but young fish of 20 mm. upwards to adult size, are found close inshore in shallow water abundantly in June and July.

It is therefore reasonable to presume that the bulk migration from deep water to warm surface water in summer is for breeding. The young as they attain 20 mm. in length along with adolescent and full grown fish with immature gonads resort to coastal waters for feeding.

## Summary

(i) Gaps have been filled in the known distribution of *Harpodon nehereus*.

(ii) The extreme limits of distribution are fixed.

(iii) Geographical and oceanographical features of the area are examined and co-relations established between them and the occurrence and abundance of the fish.

(iv) The 80° F. July isotherm and the abundance of food in the sea opposite the months of flooded rivers more than low salinity apparently provide the optimum conditions for the shoreward shoaling of 'Bombay Duck'.

(v) Estuaries and islands as additional factors influencing the abundance or absence of the fish, are discussed and a shoreward and seaward migration inferred. Additional proofs for this migration are adduced.

(vi) Gunther's theory of the deep sea origin of the 'Bombay Duck' is revived and a bathy- or mesopelagic habitat is postulated.

(vii) The primary purpose of the migration from deep water to low warm surface water is probably for spawning. The young fish of 20 mm. length and more along with full sized fish resort to the coastal waters for feeding.

## References

1. Alcock, A. .. *An. Mag. Nat. Hist.*, 1891, **2**, 128; and 1892, **2**, 356.
2. Bapat, S. V., Banerji, S. K. and Bal, D. V. "Observations on the Biology of *Harpodon nehereus* (Hamilton)," *J. Zool. Soc. India*, 1951, **3**, No. 2, 341–56.
3. Day, F. .. *Fishes of India*, 1887, p. 506, pl. CXVIII, Fig. 1.
4. Govindan, V. .. "Fishery Statistics and Information, West and East Coast of Madras Presidency," *Madras Fish. Bull.*, Madras, 1916, **9**, 1–140.

5. Good, G. B. and Bean, T. H. — *Oceanic Ichthyology*, 1895, p. 59.

6. Gunther, A. .. *Challenger Reports*, 1807, **22**,

7. ———— .. *Study of Fishes*, 1880, p. 584.

8. Hardenberg, T. D. F. .. *Treubia*, 1934, **14**, 221–22.

9. Hora, S. L. .. "Wanderings of the 'Bombay Duck', *Harpodon nehereus* in Indian waters," *J. Bomb. Nat. Hist. Soc.*, Bombay, 1934, **37**, 640–54.

10. Kemp, S. .. "Notes on the fauna of the Mutlah River in the Gangetic Delta," *Rec. Ind. Mus.*, Calcutta, 1917, **13**, 238–41.

11. Lloyd, R. E. .. "Notes on a collection of marketable fish from Akyab," *Ibid.*, Calcutta, 1907, **1**, 217–30.

12. Max Weber and Beaufort — *Fishes of the Indo-Australian Archipelago*, 1913, **2**, 151.

13. Moses, S. T. .. "A statistical account of the fish supply of Madras," *Madras Fish. Bull.*, Madras, 1922, **15**, 131–66.

14. Norman, J. R. .. *Fishes*, John Murray Expedition (1933-34), 1950, **7**, 52–53.

15. Pillai, T. V. R. .. "A preliminary note on the food and feeding habits of the 'Bombay Duck', *Harpodon nehereus* (Ham.) in the River Mutlah," *Science and Culture*, 1951, **17**, 261–62.

16. Setna, S. B. .. "Fishing for 'Bombay Duck' (*Harpodon nehereus*) Destructive Fishing Methods," *J. Bomb. Nat. Hist. Soc.*, Bombay, 1932, **35**, 867–72.

17. Sorley, H. J. .. *The Marine Fisheries of the Bombay Presidency*, Bombay (1932), 1948.

1184–54 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore

# STUDIES ON THE LIFE-HISTORY OF *TRICHURIS OVIS* (ABILDGAARD, 1795) (FAM. TRICHURIDAE: NEMATODA)

By Gobind Singh Thapar, M.Sc., Ph.D. (Lond.), F.N.I., F.A.Sc., F.Z.S.I.
AND
Kr. Suresh Singh, Ph.D. (Luck.)
*(From the Department of Zoology, University of Lucknow)*

Received on March 8, 1954

*Trichuris ovis* (Abildgaard, 1795) is a very common parasite of the domesticated animals and is found not only in the cæcum of sheep and goats in India but has also been reported from cattle and buffaloes. A light infection usually does not cause much damage but sometimes acute infections cause colitis, chronic diarrhoea and other disorders of the intestinal tract. It is also possible that these worms may cause trauma and irritation of the intestinal tract.

A large number of workers have studied the life-history of the different species of *Trichuris*. The earliest account is by Davaine (1858) who for the first time showed that the eggs of *T. trichiurus* pass out of the host unsegmented and require about 34 weeks to embryonate. Leuckart (1876) working on *T. ovis* demonstrated that, the life-history is simple and takes place by direct infection with embryonated eggs. He successfully repeated the experiments on swine by using eggs of *T. crenatus*. Railliet (1884), while working with *T. vulpis*, showed that only the embryonated eggs are infective, and non-embryonated eggs pass out without development. Grassi (1887) [quoted by Faust (1939)] for the first time described the stages in the life-history of *T. trichiurus*. Neshi (1918) [quoted by Yokogawa (1920)], working on *T. vulpis* in dogs, said that the larvæ migrate through the lungs as they do in ascarids. Tochihara (1922) working on *T. vulpis* in dogs, and Fülleborn (1923) working on *T. leporis* in rabbits, found that no migration through the lungs takes place. Hasegawa (1924), working on *T. vulpis* in dogs, found that the larvæ in the early stages migrate to the crypts of Lieberkuhn. Brown (1927) studied the rate of development of eggs in *T. trichiurus* under field conditions and found that eggs do not embryonate in direct sunlight. Spindler (1929) found that eggs of *T. vulpis* exposed to air at 77% saturation at 22° C. died in 12 days and

that there is a direct correlation between rainfall and geographical distribution of human trichurids. Hirasawa (1929) working on *T. vulpis* and *T. trichiurus* did not find any evidence of the larvæ reaching or migrating through the lungs. Nolf (1932) found that freezing temperature is lethal to the eggs of human trichurids. Matsubayashi (1932), working on *T. vulpis* in rats, followed the local migration of the larvæ in the host's intestine. Artjuch (1936) concluded that *T. ovis* enters through the crypts of Lieberkuhn and that they are blood feeders. Dinnik (1937) found that eggs of *T. trichiurus* start dividing 25–30 hours after passing out of the host and also gave the nuclear changes occurring during the early development of the eggs. Dinnik (1937, 1937 *a*, 1939) studied the development of the eggs of *T. trichiurus* under different environmental conditions and found that the egg shell can resist watery solutions of copper sulphate, hydrochloric and nitric acids, mercuric chloride and formalin. He also observed that the shell resists desiccation by an exchange of water through the polar plugs. These eggs can do without oxygen for several days during their development. Miller (1939) studied the development and hatching of eggs of *T. vulpis*. Bonilla Naar (1945) gave an account of the life-history of *T. trichiurus*. Shikhobalova and Gorodilova (1945) found that eggs of *T. trichiurus* require an average temperature of 33–35° C. and a minimum soil humidity of 8% for development. Miller (1947) worked out in detail, the early phases of the life-history of *T. vulpis* in dogs, and further confirmed the observations of Hasegawa (1924) in finding the migration of the early stages in the crypts of Lieberkuhn. Deo (1948, 1950) infected kids with embryonated eggs of *T. ovis* and recovered adult worms after 136 and 161 days respectively, but he gave no structural details. It would thus appear that no attempt has been made to give a complete and connected account of the life-history of this parasite.

In a closely allied genus, *Capillaria*, divergent views have been expressed about its life-history. Fülleborn (1924) showed that the larvæ of *C. hepatica* (syn. *Hepaticola hepatica*) migrate to the liver through the hepatic portal system and this was confirmed by Nishigori (1925), who also gave a detailed account of its life-history. When the infected liver is accidentally eaten by another host or the food is contaminated (Momma, 1930; Troisier and Deschiens, 1930; Shorb, 1931), the larvæ hatch in the intestine and migrate to the liver through the hepatic portal system and sometimes reach lung, brain, kidney or skin. Vogel (1930) showed that young larvæ of *C. hepatica*, if implanted on other organs, reach back liver. Luttermoser (1938) found that the eggs of *C. hepatica* withstand desiccation and freezing for 1–2 weeks and about 10% of eggs remain viable after 28 weeks of encapsulation in

liver, and also the larvæ penetrate the cæcum wall 6 hours after infection and reach liver *via* the hepatic portal system (Luttermoser, 1938 *a*). In some other species of *Capillaria*, the life-history may be simple and direct, as in *C. columbæ*, *C. contorta* and *C. ærophila* (Cram, 1932, 1936; Wehr, 1937; Levine, 1938; Christenson, 1938) or it may be requiring an intermediate host, as in *C. caudinflata*, *C. annulata*, *C. plica* and *C. mucronata*, for which species of earthworms are true intermediate hosts (Wehr, 1936; Allen and Wehr, 1942; Morehouse, 1944; Petrov and Borovkova, 1942; Zucchero, 1942; Wehr and Allen, 1945; Skarbilovich, 1945; Allen, 1949).

In view of these divergent views about the life-histories of the members of the family Trichuridæ and also regarding differences of opinions about migration of the larvæ through the body of the host even in *Trichuris* species, it was considered desirable to reinvestigate the life-history and the methods of infection in *T. ovis*. *The results indicate that there is neither migration through the body of the host nor is there an intermediate host involved in the life-cycle of this parasite.*

In the present work it is attempted to study the life-history of the parasite under laboratory conditions and experiments conducted to infect goats and other small laboratory animals with a view to ascertain the time taken to complete the life-history under varying conditions. Since the details of the early stages with special reference to migration have been studied by Miller (1947), greater attention had been paid in this communication to the later stages of the life-history. The present work was carried out under a grant from the Indian Council of Agricultural Research, New Delhi. Our thanks are due to the authorities of the Council for providing funds for this work. We are also thankful to the authorities of the Lucknow University for providing laboratory facilities.

## Materials and Methods

Gravid females of *T. ovis* were obtained from the local slaughter-houses and the eggs for cultures were recovered from them by cutting the body into small pieces. For final infection laboratory-reared kids were used. These animals were kept under sanitary conditions and care was taken to avoid natural infection taking place in the animals.

The earthworms for the infection experiments were reared from cocoons in the laboratory and were kept in sterile earth. They were exposed to infection according to the methods of Wehr (1936), Allen and Wehr (1942), Morehouse (1944) and Wehr and Allen (1945).

The eggs for cultures were obtained from females by teasing the uterus in a petri dish full of sterile distilled water. Care was taken to wash, as much of the debris from cultures as possible, to avoid infection by fungus and other organisms. The water of the culture was changed every day and hence there was no necessity to use dilute formalin to check the growth of fungi and bacteria. It was, however, observed that a slight infection did not hamper the development of the eggs in any way. The eggs were kept in a constant temperature cabinet at 80–82° F.

## INFECTION EXPERIMENTS

To ascertain if an intermediate host is required in the life-cycle of *T. ovis*, earthworms, *Pheretima posthuma*, *Eutyphœus waltoni* and *Allolobophora* sp. were exposed to embryonated eggs. These earthworms were examined at different intervals of time and all of them were found to be negative for larvæ and hence it was concluded that there is no intermediate host required in the life-history of *T. ovis*.

Two kids born under laboratory conditions were then selected for the final infection. These animals were not allowed to eat grass, etc., but were fed on goat's milk. The fæces of both the animals were examined regularly and it was negative for helminthic infection till the experiment started. A definite number of embryonated eggs were then fed to the kids by medicinal dropper for a number of days. The following table gives the dates and the number of eggs fed to kid A.

*Experiment I. Kid. A*

| | | |
|---|---|---|
| 3- 9–1946 .. | approx. | 200 eggs |
| 4- 9–1946 .. | „ | 200 „ |
| 5- 9–1946 .. | „ | 200 „ |
| 2–10–1946 .. | „ | 300 „ |
| 3–10–1946 .. | „ | 300 „ |
| 4–10–1946 .. | „ | 300 „ |
| TOTAL .. | | 1,500 |

During the feeding experiments a routine examination of the fæces was carried out, with negative results. However, on 22nd October 1946, *i.e.*, 7 weeks after the first feed of the eggs, non-embryonated eggs were found for the first time in the fæces. Since the number of eggs passing out at this time was very small, the kid was again given approximately 300 embryonated eggs every week. In the third week of December 1946 a large number of non-embryonated eggs were found in the fæcal examination of the kid.

In the last week of December 1946 the kid showed signs of weakness and aversion to food and eventually died on the night of December 30, 1946. The post-mortem revealed all the organs to be normal except the cæcum, which had thickened wall and contained a large number of worms in various stages of development. The following is the post-mortem report:—

| | | |
|---|---|---|
| Liver and Lungs | .. | Normal, no larvæ |
| Heart | .. | Normal, no larvæ |
| Kidneys | .. | Normal, no larvæ |
| Stomach | .. | Large for size, full. |
| Intestine | .. | Normal, free from fæcal matter |
| Abdomen | .. | Normal |
| Blood | .. | No larvæ |
| Cæcum | .. | Thickened wall, full of worms in various stages of development. |

It was, therefore, concluded that the larvæ do not enter the blood vascular system of the host and that there is no migration through different parts of the body of the host in the life-history of this parasite.

*Experiment II. Kid B*

The second kid was fed with embryonated eggs on the dates shown below:—

| | | | |
|---|---|---|---|
| 25–11–1946 | .. | approx. | 300 eggs |
| 6–12–1946 | .. | „ | 300 „ |
| 13–12–1946 | .. | „ | 300 „ |
| 20–12–1946 | .. | „ | 300 „ |
| TOTAL | .. | | 1,200 |

The fæcal examination for the non-embryonated eggs was carried out as usual after the third week of feeding and the non-embryonated eggs were seen for the first time on 16–12–1947, *i.e.*, almost 12 weeks after the first feed of the embryonated eggs was given.

The post-mortem revealed the presence of a small number of mostly mature worms.

*Experiment III. Rabbit*

To obtain some of the early stages in the life-history, a rabbit was used for final infection. About 6,000 eggs were given to the rabbit on 8–7–1949 and the rabbit was autopsied on 5–8–1949. On examination it was found that the whole of the intestinal tract was free of the larvæ but a fairly good

number of larvæ of the second stage were present in the cæcum. The larvæ were found embedded in the mucosa of the cæcum, but not very deep. Usually, the anterior region of the larva was found embedded in the mucosa and the posterior region was found hanging freely in the lumen, as is the case with mature worms. There is slight tissue damage done to the host by the embedding of the larvæ in the mucosa of the host and a thin tube-like tissue thickening is formed by the mucosa round the worm.

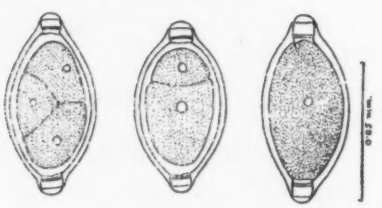
## DESCRIPTION OF THE LIFE-HISTORY

### *The Egg*

The eggs taken out of the uterus are more or less transparent though the eggs that pass out of the host along with the fæces are golden yellow in colour. With a little development the eggs become slightly yellow in colour. The eggs are barrel-shaped, provided with a plug at either end. They measure 0·071·0–078 mm. in length and 0·31–0·037 mm. in breadth, the average size being 0·074×0·033 mm.

The fresh eggs (Fig. 1) taken out of the uterus are not segmented. An unsegmented egg is uniformly filled up with numerous yolk granules. The egg-shell is thick and open at both ends where the plugs are fixed. Inside the egg-shell is a thin, vitelline membrane which covers the egg proper. Usually a narrow cavity is present all round the egg. The first division of the egg takes place 72 hours after they are taken out of the uterus. It is a transverse cleavage dividing the egg into two unequal masses or blastomeres (Fig. 2). The second cleavage is, neither transverse nor longitudinal, but more or less oblique, thus dividing the egg into three blastomeres (Fig. 3). The newly formed third cell is wedged in between the two older cells. The third cleavage occurs after 96 hours and is transverse and divides only the last formed cell thus resulting in four blastomers of the egg (Fig. 4). After

All figures drawn with the aid of Camera lucida. Figs. 1–3 are drawn to one scale; Figs, 4–6, to one scale and Figs. 7–8 to one scale.

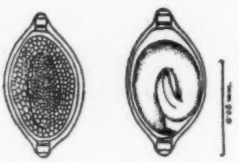


1 2 3

FIGS. 1–3. Fig. 1. Unsegmented egg; Fig. 2. First cleavage, 2 cells; Fig. 3. Second cleavage, 3 cells.

4 5 6

FIGS. 4–6. Fig. 4. Third cleavage, 4 cells; Fig. 5. Fourth cleavage, 5 cells; Fig. 6. Fifth cleavage, 6 cells.



7 8

FIGS. 7–8. Fig. 7. Morula stage; Fig. 8. With fully developed larva.

this cleavage, the egg begins to divide rather rapidly and a morula stage is reached in 5–7 days (Figs. 5–7). With the progress of the segmentation, the cells get smaller in size till the sign of the developing larva is seen as a faint demarcation running inside the morula (Fig. 7). The fully developed larva is formed in 21–22 days, but this period may extend to 35 days during the winter months provided the eggs are kept at room temperature.

*Table showing Time Required for Embryonation of Eggs**

| Date of Culture | Date of embryonation | Days |
|---|---|---|
| 3–8–1946 | 26– 8–1946 | 23 |
| 3–8–1946 | 25– 8–1946 | 22 |
| 25–8–1946 | 17– 9–1946 | 23 |
| 28–8–1946 | 17– 9–1946 | 20 |
| 11–9–1946 | 3–10–1946 | 22 |
| | AVERAGE .. | 22 |

The fully developed larva (Fig. 8) lies coiled up inside the egg-shell and shows very slight movements of coiling and uncoiling. The larvæ remain alive for a period of 6 months and hatch only when they reach the inte tine of the host. When released mechanically from the egg shell, the larva does

* The eggs were kept in a constant temperature cabinet at 80–82° F.

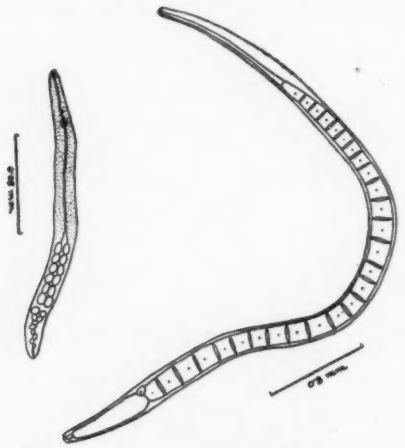
not show any active movement. Since no moulting was observed inside the egg, this larva is considered to be the first stage larva and this is also the infective stage.

*The First Stage Larva*

The first stage larva (Fig. 9) corresponds to the embryo or unhatched larva inside the egg. The fully developed larva lies coiled inside the shell and shows only slight movements and does not hatch in water, but remains alive for 24 weeks inside the egg-shell in the presence of moisture and at room temperature. When released from the egg by mechanical means, the slender larva comes out at either pole. It measures 0·125–0·15 mm. in length and 0·01–0·012 mm. in breadth (Fig. 9). It is slender elongated and tapering at both ends. At the anterior end there is a small, clear space representing the oral spear, which is usually not projecting out in the mechanically released larvæ. In larvæ, 0·148–0·15 mm. in length, the œsophagus is 0·1–0·12 mm. in length and possesses a slight swelling in the head region and also just anterior to the beginning of the cell body. The cell body is about 0·042–0·06 mm. in length and consists of two rows of rounded and granular cells. The intestine is about 0·018–0·02 mm. in length and consists of a few cells. The rectum is 0·098 mm. in length and appears as a narrow tube.

*The Second Stage Larva*

In the second stage larvæ, intestine becomes comparatively long (Fig. 10). The stylet or spear is absent, though in one specimen, it was seen projecting



9 10

FIGS. 9 & 10. Fig. 9. First stage larva mechanically released. Fig. 10. Second stage larva, entire.

out of the oral end (Fig. 11). In a specimen measuring 2·15 mm. in length the œsophagus measures 1·75 mm. in length and the intestine 0·28 mm.

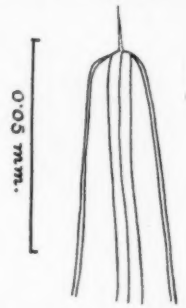



FIG. 11. Second stage larva showing oral spear.

in length. The cell body consists of a single row of cells with prominent nuclei. The rectum is still short but has become considerably wider. The anus is subterminal. This larva was recovered after 28 days of infection. The two cells of uncertin nature and origin are present at the œsophago-intestinal junction. In one larva which was recovered along with this larva, the ratio of the œsophagus and the intestine was the same as in the first stage larva, though structurally it belonged to the second stage. This larva measures 4·9 mm. in length and the œsophagus and the intestine measure 4·1 mm. and 0·66 mm. respectively. The rectum measures 0·085 mm. in length.

*The Third Stage Larva*

In the third stage larva no structural difference could be seen from the second stage larva except the presence of the genital primordium. The larva is more slender and elongated and the intestine is comparatively longer (Figs. 12, 13, 14). In a larva measuring 6·5 mm. in length, the œsophagus

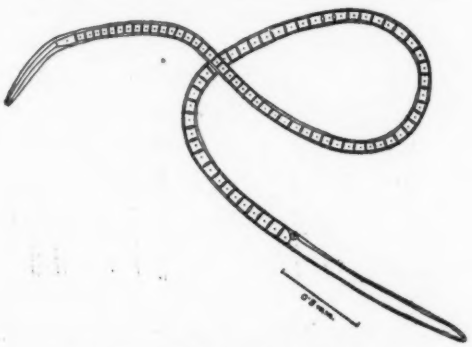
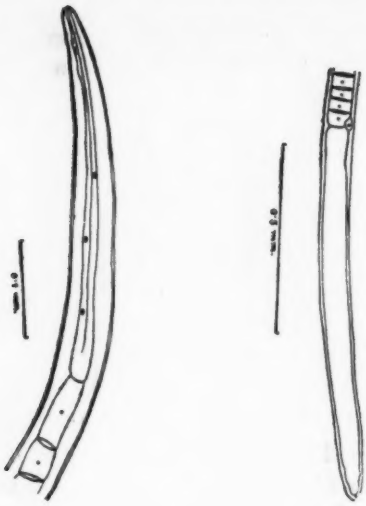


FIG. 12. Third stage larva, entire.



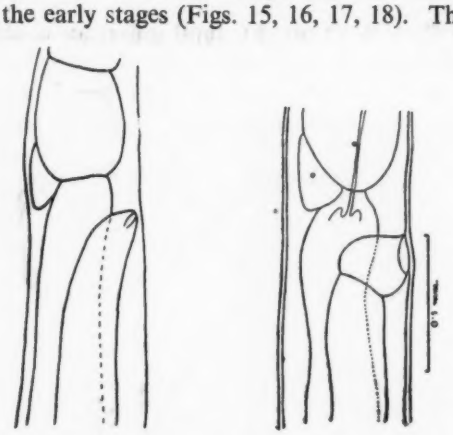
13 14

FIG. 13. Third stage larva, anterior end.
FIG. 14. Third stage larva, posterior end.

measures 5·3 mm. in length and the intestine 1·1 mm. in length. The rectum is 0·1 mm. in length. This larva was recovered 7 weeks after infection.

*The Fourth Stage Larva*

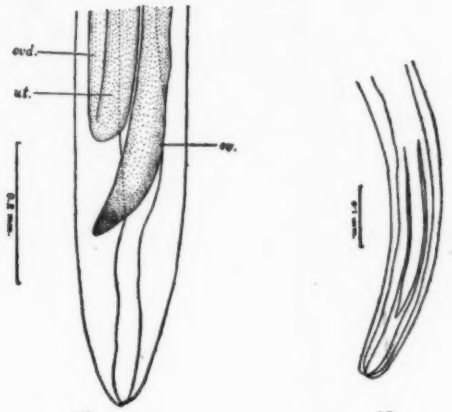
The fourth stage larva is well developed and the sexes can be distinguished even in the early stages (Figs. 15, 16, 17, 18). The larvæ measure



15 16

FIG. 15. Fourth stage larva, early, female genitalia.
FIG. 16. Fourth stage larva, late, female genitalia.

14·1–23 mm. in length. In one specimen, measuring 23 mm. in length the œsophagus is about 16·1 mm. in length and the intestine is 5·4 mm. in length.




17 18

FIG. 17. Fourth stage larva, late female genitalia.
FIG. 18. Fourth stage larva, early, male genitalia.

The female organs are represented by an ovary, uterus, oviduct and the vagina. The vulva is represented by a concavity in the body-wall in the region of œsophago-intestinal junction in early stages. In some specimens the vulva is not in communication with the vagina. The vagina runs back for a short distance and imperceptibly passes on into the uterus which extends up to the posterior region. The oviduct runs parallel to the uterus and is reflexed back to join the ovary, which extends upto the posterior end.

The single testis runs forward from the junction of the intestine with the rectum and on reaching the œsophago-intestinal junction reflexes back and continues as the vas deferens which opens into a comparatively wide ductus ejaculatorius. The weakly cuticularised spicule is present and shows very small processes on the sheath which later on form the spines (Fig. 19). The spicule in the late fourth stage is about 1·3 mm. in length. The spicular sheath is present around the spicule.

*Adults*

The characters distinguishing the various species of the genus *Trichuris* are not well pronounced and it has always been difficult to give clear diagnosis. Schwartz (1926) and Baylis (1929) used the size of spicules, structure of the spicular sheath and size of eggs to differentiate the species. Chandler

(1930) used the length of spicules in conjunction with other characters such as length of cloacal tube, length and shape of the ejaculatory duct and the nature of testis and vas deferens. Bhalerao (1935) considered the length of

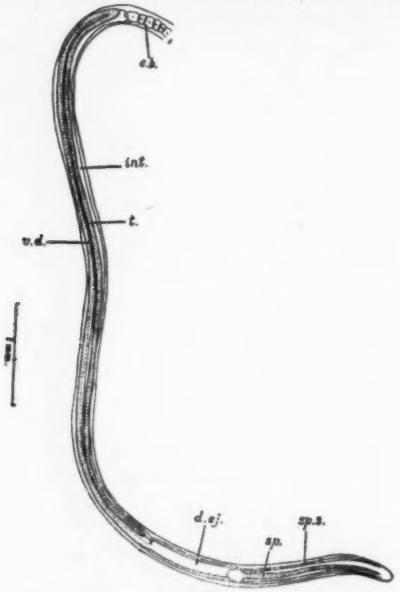



FIG. 19. Fourth stage larva, late, male genitalia.

œsophagus, length of spicules, nature of spicular sheath and size of eggs for diagnosis. Artjuch (1936) showed that the nature of the spicular sheath changes with the physiological conditions and hence is unreliable. Clapham (1945) supported Chandler's view and suggested that the nature of vulva may be used in case only females are available for study. It was also suggested by Clapham that the character of the head and œsophagus should be considered as these characters are common to both the sexes. Sarwar (1945, 1945 *a*, 1946) pointed out the unsatisfactory systematics of the species of *Trichuris* and without clearing the position, described two new species from India. Tiner (1950) used the size of spicules and eggs, and the relative lengths of the vas deferens and ejaculatory duct and œsophagus and intestine. In the present communication we have mainly relied on the comparative length of œsophagus and intestine, the size of spicule, the nature of spicular sheath, the length of cloacal tube, vas deferens and ejaculatory duct, nature of vagina and the size of eggs, to differentiate the species.

The following is a short redescription of *T. ovis* based on a large number of specimens collected at Lucknow.

*Male.*—Length 58–82 mm., the anterior thin region is about 1½ times longer than the broad posterior region. Oesophagus is 4–5 : 3 times longer than the intestine. In one specimen 58 mm. long, the œsophagus is 36 mm. and the intestine is 21 mm. in length. Oesophagus is 0·077 mm. in diameter and the intestine is 0·124 mm. in diameter. Spicule is 4·4–6 mm. in length. Spicular sheath is spiny along the entire length, the spines on the expansion being shorter than the rest. The bulb may be present or absent. The spicular sheath and the bulb are completely retractile. The cloacal tube is 2–4 mm. long. The ejaculatory duct is 8–9·5 mm. long and the vas deferens is 6–7·5 mm. long.

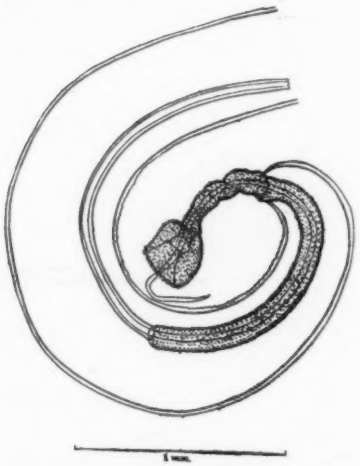


FIG. 20. Adult male, posterior end.

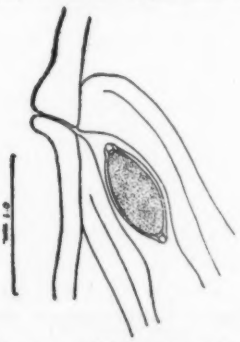


FIG. 21. Adult female, vulva.

*Statement showing incidence and intensity of* Trichuris ovis *in sheep and goat*
For the years 1939-41

| | April | | | | | May | | | | | June | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal |
| SHEEP | | | | | | | | | | | | | | | |
| 1939-40 .. | 22 | 16 | 72·7 | 103 | 6 | 24 | 18 | 75·0 | 188 | 10 | 25 | 15 | 60·0 | 132 | 9 |
| 1940-41 .. | 25 | 24 | 96 | 586 | 24 | 26 | 25 | 96·1 | 457 | 18 | 24 | 24 | 100·0 | 423 | 17 |
| GOAT | | | | | | | | | | | | | | | |
| 1939-40 .. | 22 | 16 | 72·6 | 129 | 8 | 24 | 15 | 62·5 | 151 | 10 | 25 | 15 | 60·0 | 152 | 10 |
| 1940-41 .. | 25 | 23 | 92·0 | 496 | 21 | 25 | 25 | 100·0 | 361 | 14 | 24 | 24 | 100·0 | 436 | 18 |

| | July | | | | | August | | | | | September | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal |
| SHEEP | | | | | | | | | | | | | | | |
| 1939-40 .. | 23 | 17 | 74·0 | 195 | 11 | 24 | 19 | 82·5 | 419 | 22 | 23 | 20 | 87·0 | 390 | 19 |
| 1940-41 .. | 27 | 27 | 100·0 | 908 | 34 | 25 | 25 | 100·0 | 871 | 35 | 24 | 24 | 100·0 | 614 | 26 |
| GOAT | | | | | | | | | | | | | | | |
| 1939-40 .. | 23 | 18 | 81·8 | 169 | 9 | 24 | 20 | 83·3 | 232 | 12 | 23 | 19 | 82·6 | 230 | 12 |
| 1940-41 .. | 27 | 27 | 100·0 | 617 | 23 | 25 | 25 | 100·0 | 837 | 33 | 24 | 24 | 100·0 | 557 | 23 |

| | October | | | | | November | | | | | December | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal |
| SHEEP | | | | | | | | | | | | | | | |
| 1939–40 .. | 24 | 22 | 91·7 | 229 | 10 | 23 | 21 | 91·3 | 178 | 8 | 22 | 21 | 95·4 | 231 | 11 |
| 1940–41 .. | 19 | 18 | 94·7 | 280 | 15 | 23 | 22 | 95·6 | 199 | 9 | 23 | 21 | 91·3 | 127 | 6 |
| GOAT | | | | | | | | | | | | | | | |
| 1939–40 .. | 23 | 18 | 81·8 | 142 | 8 | 23 | 19 | 82·6 | 122 | 6 | 22 | 20 | 91·0 | 171 | 9 |
| 1940–41 .. | 19 | 19 | 100·0 | 302 | 16 | 23 | 16 | 69·6 | 186 | 12 | 23 | 21 | 91·3 | 160 | 9 |

| | January | | | | | February | | | | | March | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal | No. Examined | No. Infected | % of Infection | Total No. of parasites recovered from the infected animals | Average load per animal |
| SHEEP | | | | | | | | | | | | | | | |
| 1939–40 .. | 25 | 25 | 100·0 | 1234 | 49 | 22 | 22 | 100·0 | 843 | 38 | 21 | 20 | 95·2 | 659 | 33 |
| 1940–41 | 23 | 21 | 91·3 | 414 | 20 | 16 | 16 | 100·0 | 184 | 11 | 22 | 16 | 72·7 | 199 | 12 |
| GOAT | | | | | | | | | | | | | | | |
| 1939–40 .. | 25 | 25 | 100·0 | 570 | 23 | 22 | 22 | 100·0 | 477 | 22 | 21 | 21 | 100·0 | 450 | 21 |
| 1940–41 .. | 23 | 20 | 87·0 | 277 | 14 | 16 | 15 | 93·7 | 147 | 10 | 22 | 18 | 81·7 | 237 | 13 |

UNIVERSITY OF MICHIGAN LIBRARIES

*Female.*—Length 63–80 mm. and the thin anterior region is 2½–3 times longer than the broad posterior region. The approximate ratio of the length of the œsophagus to intestine is 5–5·5 : 2. In one specimen measuring 53 mm. in length, the œsophagus is 36 mm. and the intestine is 14 mm. long. Oesophagus is 0·077 mm. and the intestine 0·124 mm. in diameter. The vagina is long, muscular and wavy with no sudden expansion forming the egg chamber. The vulva is present just posterior to the junction of the œsophagus and the intestine. Eggs measure 0·071–0·078 mm. in length and 0·031–·037 mm. in breadth, the average size being 0·074×0·033 mm. The eggs are provided with two polar plugs.

## INCIDENCE AND INTENSITY OF INFECTIONS OF *Trichuris ovis*

*Trichuris ovis* is found in the large intestine and cæcum of sheep and goats. It has also been reported from cattle and buffaloes but the infection in these is very small and negligible. As the infection in sheep and goat is very heavy, it was decided to study it by regular examination of one animal of each type selected at random daily at the slaughter-houses in Lucknow. The results are embodied in a table for 2 years, 1939–41. The observations indicate that the percentage of infection in both sheep and goats is same, there being very slight variations. Besides, the table indicates constant seasonal variations in the incidence and intensity of infection in both sheep and goats.

The ratio of male and female worms in sheep and goats were determined at Lucknow and the results are indicated in the following table:

| | Total Number of Male recovered | Total Number of Female recovered | Ratio between Males & Females |
|---|---|---|---|
| Sheep .. | 610 | 1,481 | 2 : 5 |
| Goat .. | 361 | 889 | 2 : 5 |

## SUMMARY

1. It has been confirmed that the life-history of *Trichuris ovis* is simple and direct and no migration of the larvæ takes place in the body of the host and no intermediate host is involved in the process.

2. Segmentation of egg under laboratory conditions has been described upto the formation of the embryo or the first stage larva.

3. The various stages of larval development, from the first to the fourth stage have been studied.

4. The first stage larva is characterised by the presence of an oral spear and a double row of cells in the cell body. The approximate ratio of

œsophagus and intestine is 7 : 1. In later stages, the oral spear is lost and the cell body consists of a single row of cells, with prominent nuclei.

5. In the second stage larva, the ratio of œsophagus and intestine is reduced to 6 : 1, though sometimes it is slightly greater.

6. The third stage larva shows the presence of genital rudiments and the ratio of the œsophagus and the intestine becomes 5 : 1.

7. In the fourth stage larva, the sexes can be differentiated and in its later stages, almost all the sexual organs can be distinguished. The ratio of œsophagus and intestine becomes 3 : 1.

## References


Allen, R. W. .. "Studies on the life-history of *Capillaria annulata* (Molin, 1858) Cram, 1926," *Jour. Parasit.*, **35**, *Suppl.*, 1949, 35.

—— and Wehr, E. E. .. "Earthworms as possible intermediate hosts of *Capillaria caudinflata* of the chicken and turkey," *Proc. Helm. Soc. Wash.*, 1942, **9**, 72–73.

Artjuch, E. .. "Uber die Verbeitung der Trichocephaliden der Schafe in U.d.S.S.R. und ihre Bedentung under Zugrundnahme ihrer Fixationsweise und Ernahrungsart," *Wiss. Ber. Tier. Zoo. Hoch. Wit.*, (U.d.S.S.R.), 1936, **4**, 107–114.

Bancroft, T. L. .. "On the whipworm of the rats liver," *Jour. Proc. Roy. Soc., New South Wales*, 1893, **27**, 86–90.

Baylis, H. A. .. *A Manual of Helminthology, Medical and Veterinary*, 1929, London.

Bhalerao, G. D. .. "Helminth Parasites of Domesticated Animals in India," *Ind. Council Agr. Res., Sci. Mem.*, 1935, No. 6.

Brown, H. W. .. "Studies on the rate of development and viability of the eggs of *Ascaris lumbricoides* and *Trichuris trichiura* under field conditions," *Jour. Parasit.*, 1927, **14**, 1–15.

Bonilla Naar, A. .. "Ciclo evolutive de'l *Trichocephalus trichiurus*." *Rev. Fac. Med. Bagota*, 1945, **13**, 724.

Chandler, A. C. .. "Specific Characters in the genus *Trichuris* with a description of a new species, *Trichuris tenuis*, from a camel," *Jour. Parasit.*, 1930, **16**, 198–206.

Christenson, R. O. .. "Life-history and epidemiological studies on the fox lungworm, *Capillaria ærophila* (Creplin, 1839)." *Liv. Jub. Prof. L. Travassos*, Rio de Janeiro, 1938, 119–136.

Clapham, P. A. .. "On some characters of the genus *Trichuris* and a description of *T. parvispicularis* n. sp. from a Cane rat," *Jour. Helm.*, 1945, **21**, 85–89.

Cram, E. B. .. "In *Bobwhite Quail*," New York. 1932.

——— .. "Species of *Capillaria* Parasitic in the upper digestive tract of birds," *U.S. Dept. Agri. Tech. Bull.*, 1936, No. 516, 1–27.

Davaine, M. C. .. "Recherches sur le development et la propagation du Trichocephala de l'Homme et de *l'Ascaride lombricoide*," *Compt. Rend.*, 1858, **46**, 1217–1219.

Deo, P. G. .. "On the life-history of *Trichuris ovis* with experiments on the embryonation and extracorporeal hatching of the eggs of *Trichuris* sp.", *Proc. Ind. Sci. Congress*, 1948, **34**, 1947, Part III (Abstracts), 173.

———— .. "Studies on the life-history of *Trichuris ovis*," *Ibid.*, 1950, **37**, 1950, Part III, (Abstracts), 243–244.

Dinnik, J. A. and Dinnik, N. N. "Influence de la temperature de l'absence d'oxygene et du dessechment sur les œufs de *Trichocephalus trichiurus* (L.)," *Med. Parasit. & Parasitic Diseases*, 1937, **6**, 603–617. (In Russian: French Summary).

Dinnik, N. N. .. "On the sexual processes in the egg of *Trichocephalus trichuris* after their elimination from the host," 30*th Jub. K. J. Skjrabin*, 1937, 129–131.

Dinnik, Y. A. & Dinnik, N. N. "The structure of the shell and resistance of the eggs of *Trichocephalus trichiurus*," *Ibid.*, 1937, 132–138.

———— .. "Observations sur les development des œufs du *Trichocephalus trichiurus* (L.) dans le sol," *Med. Parasit. & Parasitic Diseases*, 1939, **8**, 221–228.

Efremor, V. V. and Shikhobalova, N. P. "Histopatogenese de la Trichocephalose experimentale chez les souris blanches," *Ibid.*, 1939, **8**, 81–88. (In Russian: French Summary).

Faust, E. C. .. *Human Helminthology*, 2nd Edition. Philadelphia, 1939.

Fülleborn, F. .. "Ueber die Entwicklung von *Trichozephalus* in wirte," *Arch. Schiffs. u. Tropen-Hyg.*, 1923, **27**, 413–420.

———— .. "Ueber den Infektionsweg bei *Hepaticola hepatica*," *Ibid.*, 1924, **28**, 48–61.

Hall, M. C. .. "Nematode Parasites of mammals of the orders Rodentia, Lagomorpha, and Hyracoidea," *Proc. U.S. Nat. Mus.*, 1916, **50**, 30–33.

Hasegawa, T. .. "Beitrag sur Entwicklung von *Trichozephalus* in wirte," *Arch. Schiffs. u. Tropen-Hyg.*, 1924, **28**, 337–340.

Hirasawa, .. "Experimental studies on the migration and development of the parasites in *Trichocephalus* infection." *Kumamoto Med. Jour.*, 1929, 5.

Leuckart, R. .. *Die Menschlichen Parasiten und die von ihnen Herruhrenden Kankheiten*, 2nd Edition, Leipzig & Heidelberg, 1876.

Levine, P. P. .. "A new method for embryonating nematode eggs in fæcal discharges." *Jour. Parsit.* (*Research Notes*), 1936, **22**, 291.

———— .. "The effect of various environmental conditions on the viability of the ova of *Capillaria columbæ* (Rud.)," *Ibid.* (*Research Notes*), 1937, **23**, 427–428.

———— .. "Infection of the chicken with *Capillaria columbæ* (Rud.)," *Ibid.*, 1938, **24**, 45–52.

Li, H. C. .. "On the mouth-spear of *Trichocephalus trichiuris* and of a *Trichocephalus* sp. from a monkey, *Rhesus macacus*," *Chin. Med. Jour.*, 1933, **47**, 1343–1346.

Luttermoser, G. W. .. "Factors influencing the development and viability of the eggs of *Capillaria hepatica*." *Amer. Jour. Hyg.*, 1938, **27**, 275–289.

Luttermoser, .. "An experimental study of *Capillaria hepatica* in the rat and the mouse," *Ibid.*, 1938*a*, **27**, 321–340.

Matsubayashi, H. .. "On the development of *Trichuris* of dog in the intestine of rats," *Keio–Igaka*, 1932, **12**, 1081–1091.

Miller, F. H. .. "Hæmorrhagic colitis of the dog due to infection with the *Trichocephalus depressiusculus*," *Amer. Vet. Rev.*, 1904, **28**, 722–729.

Miller, M. J. .. "*Trichocephalus* and Trichocephaliasis," *Can. Jour. Comp. Med.*, 1939, **3**, 282–287.

———— .. "Studies on embryonation and hatching of the eggs of the dog whipworm *Trichocephalus vulpis*," *Proc. Soc. Exp. Biol. Med.*, 1939*a*, **42**, 303–305.

———— .. "Studies on the life-history of *Trichocephalus vulpis* the whipworm of dogs," *Can. Jour. Res.*, D. 1947, **25**, 1–11.

Momma, K. .. "Notes on modes of rat infestation with *Hepaticola hepatica*," *Ann. Trop. Med. Parasit.*, 1930, **24**, 109–113.

Morehouse, N. F. .. "Life-cycle of *Capillaria caudinflata*, a nematode parasite of the common fowl," *Iowa State Coll. Jour. Sci.*, 1944, **18**, 217–253.

Neshi, G. .. "On the development of *Trichocephalus* in the body of the host," *Tokyo Ijji Sinshi*, 1918, 2080.

Newbigin, H. F. and Morgan, D. O. "The effect of certain dressings on worm-infested poultry runs," *Scot. Jour. Agri.*, 1936, **19**, 162–166.

Nishigori, M. .. "On the life-history of *Hepaticola hepatica* (Second Report)," *Jour. Formosa Med. Assn.*, 1925, No. 247, 908–919.

Nolf, L. O. .. "Experimental studies on certain factors influencing the development and viability of the ova of the human *Trichuris* compared with those of the human *Ascaris*," *Amer. Jour. Hyg.*, 1931, **16**, 288–322.

Petrov, A. M. and Borovkova, A. M. "Contribution to the study of the cycle of development of *Capillaria plica* (Rudolphi, 1819), a nematode of the bladder of dog and fox," *Compt. Rend.* (*Dokl.*) *de l'Acad. Sci. l'URSS.*, 1942, **35**, 159–160.

Railliet, M. .. "Development Experimentales du Trichocephale du Chien. Notices Helminthologiques," *Bull. Soc. centrale med. vet*, 1884, 449–452.

———— .. "Recherces Experimentales sur les Tumeurs Vermineuses du Foie des Murides," *Bull. Soc. Zool.*, 1889, **14**, 62–67.

Sarwar, M. M. .. "Incidence of some nematodes of domestic ruminants in the Punjab and United Provinces with a note on the morphology of *Trichuris globulosa* (V. Linstow)," *Proc. Ind. Acad. Sci.*, Sec. B, 1945, **22**, 274–278.

———— .. "Two new records and a new species of the genus *Trichuris* from domestic ruminants," *Curr. Sci.*, 1945*a*, **14**, 306–307,

———— .. "A new species of genus *Trichuris* from cattle and buffaloes," *Ibid.*, 1946, **15**, 52–53.

Schwartz, B. .. "Specific identity of whipworms from swine," *Jour. Agri. Res.*, 1926, **33**, 311–316.

Shikhobalova, N. P. and Gorodilova, L. I. "Dependence of the small percentage of ascariasis and trichuriasis in Samarkand on climatic and microclimatic factors," *Med. Parasit. & Parasit. Diseases*, 1945, **14**, 23-30 (in Russian).

Shorb, D. A. .. "Experimental infestation of white rats with *Hepaticola hepatica*," *Jour. Parasit.*, 1931, **17**, 151–154.

Skarbilovich, T. S. .. "A contribution to the knowledge of the biological cycle of *Capillaria mucronata* (Molin, 1858) a nematode of the bladder of sable, marten and mink," *Comp. Rend.* (*Dokl.*) *de l'Acad. Sci. de l'URSS.*, 1945, **48**, 687–88.

Spindler, L. A. .. "A study of the temperature and moisture requirements in the development of the eggs of the dog trichurid (*Trichuris vulpis*)," *Jour. Parasit.*, 1929, **16**, 41–46.

——— .. "The relation of moisture to the distribution of human trichuris and ascaris," *Amer. Jour. Hyg.*, 1929*a*, **10**, 476–496.

Tiner, J. D. .. "Two new species of *Trichuris* from North America with redescriptions of *Trichuris opaca* and *Trichuris leporis* (Nematoda : Aphasmidia)," *Jour. Parasit.*, 1950, **36**, 350–355.

Tochihara, T. .. "Studies on the development of the whipworm," *Saikingaku Zassi*, 1922, 327 (In Japanese).

Troisier, J. and Deschiens, R. "L'Hepaticoliase," *Ann. Med.*, 1930, **27**, 414–425.

Vogel, H. .. "Ueber die Organotropie von *Hepaticola hepatica*," *Ztschr. Parasitenk.*, 1930, **2** (4), 502–505.

Wehr, E. E. .. "Earthworms as transmitters of *Capillaria annulata* the 'crop-worm' of chickens," *N. Amer. Vet.*, 1936, **17**, 18–20.

——— .. "Studies on the development of the Pigeon Capillarid, *Capillaria columbæ*," *Jour. Parasit.*, 1937, **23**, 573.

——— .. "Studies on the development of the Pigeon Capillarid *Capillaria columbæ*," *U.S. Dept. Agri. Bull.*, 1939, **679**, 1–19.

——— and Allen, R. W. .. "Additional studies on the life-cycle of *Capillaria caudinflata*, a nematode parasite of chickens and turkeys," *Proc. Helm. Soc. Wash.*, 1945, **12**, 12–14.

Yokogawa, S. .. "On the migratory course of *Trichosomoides crassicauda*, (Bellingham) in the body of the final host," *Jour. Parasit.*, 1920, **7**, 80–84.

Zucchero, P. T. .. "Notes on the life-cycle of *Capillaria annulata*," *Proc. West Virginia Acad. Sci.*, 1942, **15**, 96-106.

## ABBREVIATIONS

*c.b.*, cell body; *d.ej.*, ductus ejaculatorius; *int.*, intestine; *ov.*, ovary; *ovd.*, oviduct; *sp.*, spicule; *sp.s.*, spicular sheath; *t.*, testis; *ut.*, uterus; *v.d.*, vas deferens.

1293–54 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent and Published by The Indian Academy of Sciences, Bangalore

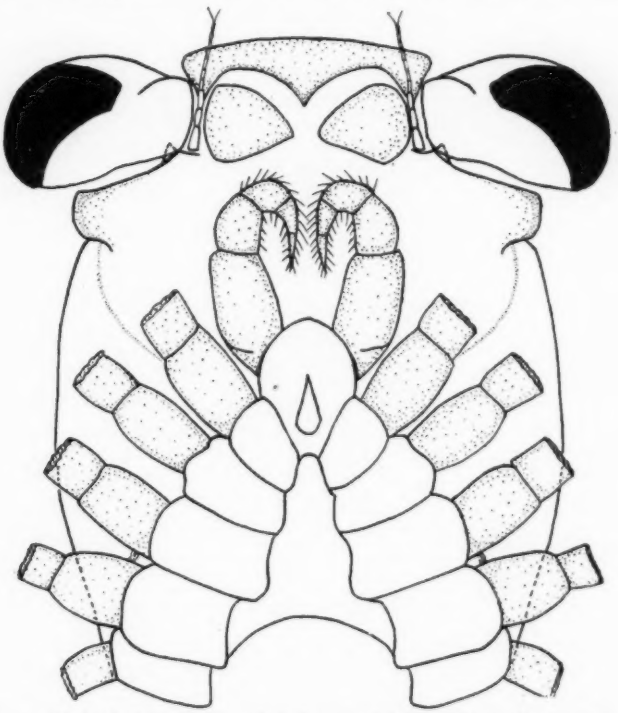
# THE POST-LARVAL DEVELOPMENT OF THE SHORE CRAB *OCYPODA PLATYTARSIS* M. EDWARDS AND *OCYPODA CORDIMANA* DESMAREST

By Miss K. G. Raja Bai Naidu, M.Sc.
*(Research Scholar, Andhra University, Waltair)*

Received July 14, 1953
(Communicated by Professor R. V. Seshaiya, F.A.SC.)

## Previous Work

In the family Ocypodidæ the megalopæ have been described only in the two genera *Uca* and *Ocypoda*. Say (1817) described a new genus and species *Monolepis inermis* which he referred to Macrura though he was aware of its Brachyuran features. Dana (1852) described and figured



Text-Fig. 1. Ventral view of the cephalothorax of the megalopa of *Ocypoda platytarsis*.

a second species *Monolepis orientalis* from Philippines. Smith (1880) definitely established the identity of the forms described by Say and Dana as the megalopa of *Ocypoda quadrata* after an examination of the young crab within the partially cast skin of one of the megalopæ collected by him at New England and Long Island which buried itself in sand and tried to moult. In 1915 Kemp studied the habits of the megalopæ of *Ocypoda macrocera* in the sandy beach of Ennur near Madras. Rathbun (1924) got a megalopa from Galapagos and identified it as belonging probably to *O. gaudichaudi*. This megalopa was later identified by Crane (1940) as belonging to the genus *Plagusia*. Lebour (1932) referred two megalopæ collected at Philippines provisionally to the family Ocypodidæ. Crane (1940) considers one of these as an *Ocypoda*, and the second with seven hairs on the last pleopod and with no hairs between the third and the fourth pair of legs as not an *Ocypoda*. Crane (1940) has also described the zoeæ and megalopæ of *Ocypoda gaudichaudi* Milne Edwards and the megalopa of *O. occidentalis* Stimpson which are different from the megalopa of the common *O. albicans* (Bosc) as shown by the number of setæ on the last pleopod.

## I. MEGALOPA AND FIRST CRAB STAGE OF *Ocypoda platytarsis* (Figs. 1–17)

### *Megalopa*

Twelve megalopæ of *Ocypoda platytarsis* were collected on Waltair beach on 12–12–1951 while they were in the act of burrowing into sand during high tide (Fig. 1).

Four of these were placed in separate finger bowls with sand and sea water, and fed on crab muscles, shrimps and mantle pieces of bivalves. Three lived for about 3 to 4 days while the fourth moulted into the first crab stage on 18–12–1951. The megalopa skin broke open at the posterior end of the cephalothorax and the first crab stage emerged. On 29–12–1951 it was killed and preserved.

Subsequently during the period, April to July 1952, fourteen megalopæ were collected. Though attempts were made to rear them, none moulted and all died within a week.

On 12–2–1953 five megalopæ were collected from burrows of about six inches depth and 3/16 inch diameter, about 25 feet away from the sea in the coastal battery region of Vizagapatam. Later many more were collected in burrows in the same region.

*Colouration.*—Carapace is yellowish-grey or light bluish-grey. The frontal and gastric regions are white or light blue or it may have the same colouration as the rest of the carapace. The lateral and posterior regions of the carapace are either yellow or pale grey. Chelipeds and ambulatory legs are ash or light brown with tiny black chromatophores.

The megalopæ have been observed to undergo changes in colour when kept in the laboratory. These colour changes take place in about 2 to 3 days.

The carapace of the cast skin of the megalopa is 7 mm. in length and 6 mm. in width at the broadest part. The front end of the carapace forms a three-horned rostrum of which the central one is the largest (Fig. 2). The rostrum is deflexed to the antero-ventral side and is therefore not visible dorsally.

The carapace is without pores. In the suborbital region the carapace shows on either side a club-shaped prominence followed by a constriction. The postero-lateral regions of the carapace show on either side an indentation for the reception of the last pair of legs. The lateral margins of the carapace are deep and vertical and serve for the reception of the first, second and third pair of ambulatory legs. Hooks are absent on the ischium of the legs. There is a tuft of hairs between the bases of second and third pair of ambulatory legs—a characteristic feature of the *Ocypoda*. The dactyli of last pair of legs have three long feelers.

*Appendages.*—Eye is well developed, stalked and projecting but without horns.

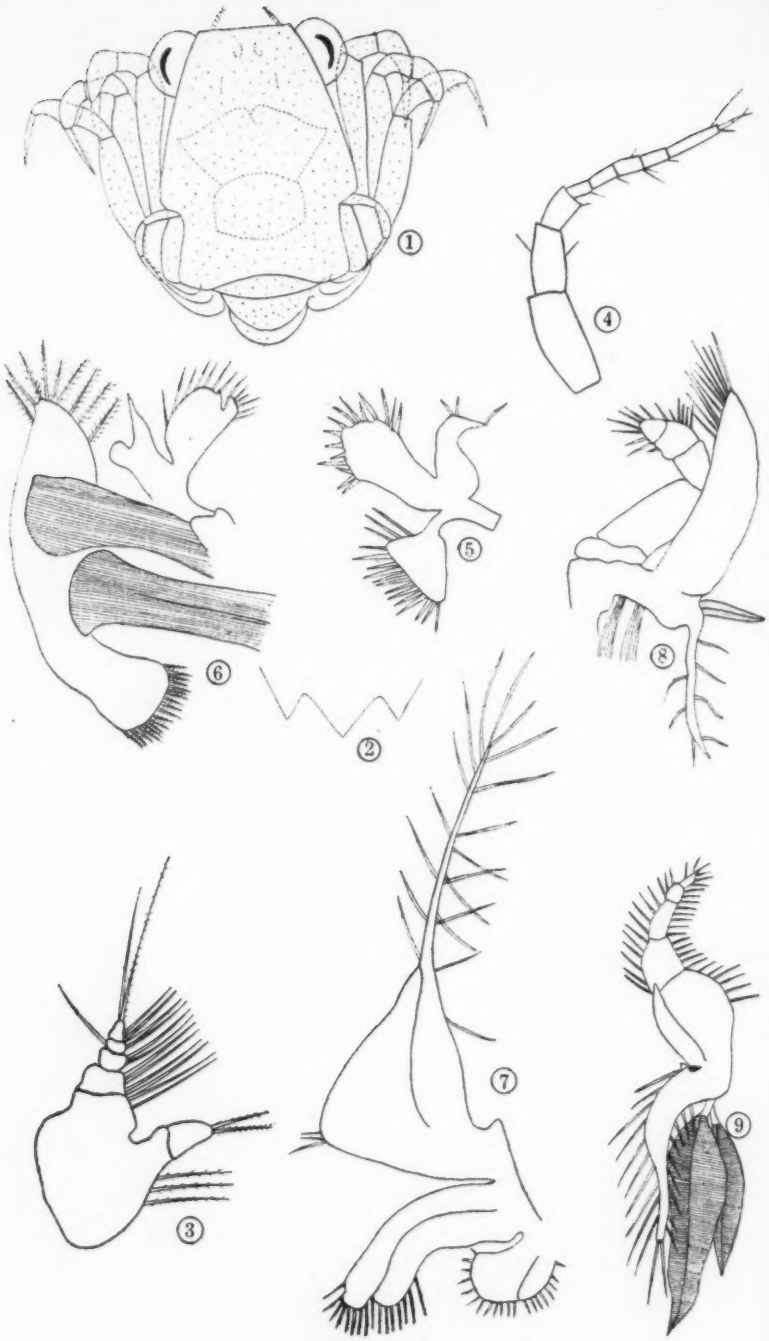
*First Antenna.*—The first antenna consists of a large basal segment containing the statocyst, and a five-jointed process having two long setæ at the distal end (Fig. 3). The flagellum is given off from the first segment and terminates in two setæ. All the segments are fringed on the inner side with a number of long setæ. The fourth segment has one seta on the outer side.

*Second Antenna* (Fig. 4).—Consists of three basal segments and a flagellum of seven segments. One or two sensory hairs are present on all segments except the proximal basal segment.

*Mandible* is well developed with a cutting process and a palp.

*First Maxilla* (Fig. 5).—It consists of a coxopodite, a basipodite and an endopodite.

*Second Maxilla* (Fig. 6).—Consists of a protopodite, an endopodite and an exopodite. The protopodite is composed of a short coxopodite and




FIGS. 1–9

FIGS. 1–9. Fig. 1. Megalopa of *Ocypoda platytarsis*, × 10. Fig. 2. Rostrum of *O. platytarsis*—front view, × 10. Fig. 3. Palp of first antenna of the megalopa of *O. platytarsis*, × 100. Fig. 4. Second antenna of the megalopa of *O. platytarsis*, × 15. Fig. 5. First maxilla of the megalopa of *O. platytarsis*, × 40. Fig. 6. Second maxilla of the megalopa of *O. platytarsis*, × 40. Fig. 7. First maxillipede of the megalopa of *O. platytarsis*, × 15. Fig. 8. Second maxillipede of the megalopa of *O. platytarsis*, × 40. Fig. 9. Third maxillipede of the megalopa of *O. platytarsis* × 15.

(*N.B.*—Figures have been reduced to $^{11}/_{25}$ the magnification given along with the explanation of figures.)

a distal basipodite; the latter is partially divided into two lobes, fringed with setæ. The endopodite is on the outer side of basipodite. The scaphognathite is broad and is fringed with setæ at the anterior and posterior margin.

*First Maxillipede* (Fig. 7).—The protopodite consists of the usual two parts, a coxopodite and a basipodite. The coxopodite and basipodite are clothed with a number of setæ. The endopodite and exopodite are armed with setæ at the distal ends. The epipodite is well developed.

*Second Maxillipede* (Fig. 8).—The protopodite is very much reduced. The endopodite is comparatively larger than that of the first maxillipede. It arises from the basipodite and is divided into five parts. The proximal most segment is the basischiopodite. The other segments are meropodite, carpopodite, propodite and dactylopodite. The exopodite and the epipodite are in the same position as in the first maxillipede but the epipodite is much shorter than that of the first maxillipede.

*Third Maxillipede.*—It is built on the same plan as the second maxillipede (Fig. 9).

The mandible, maxillæ and maxillipedes are exactly like those of an adult.

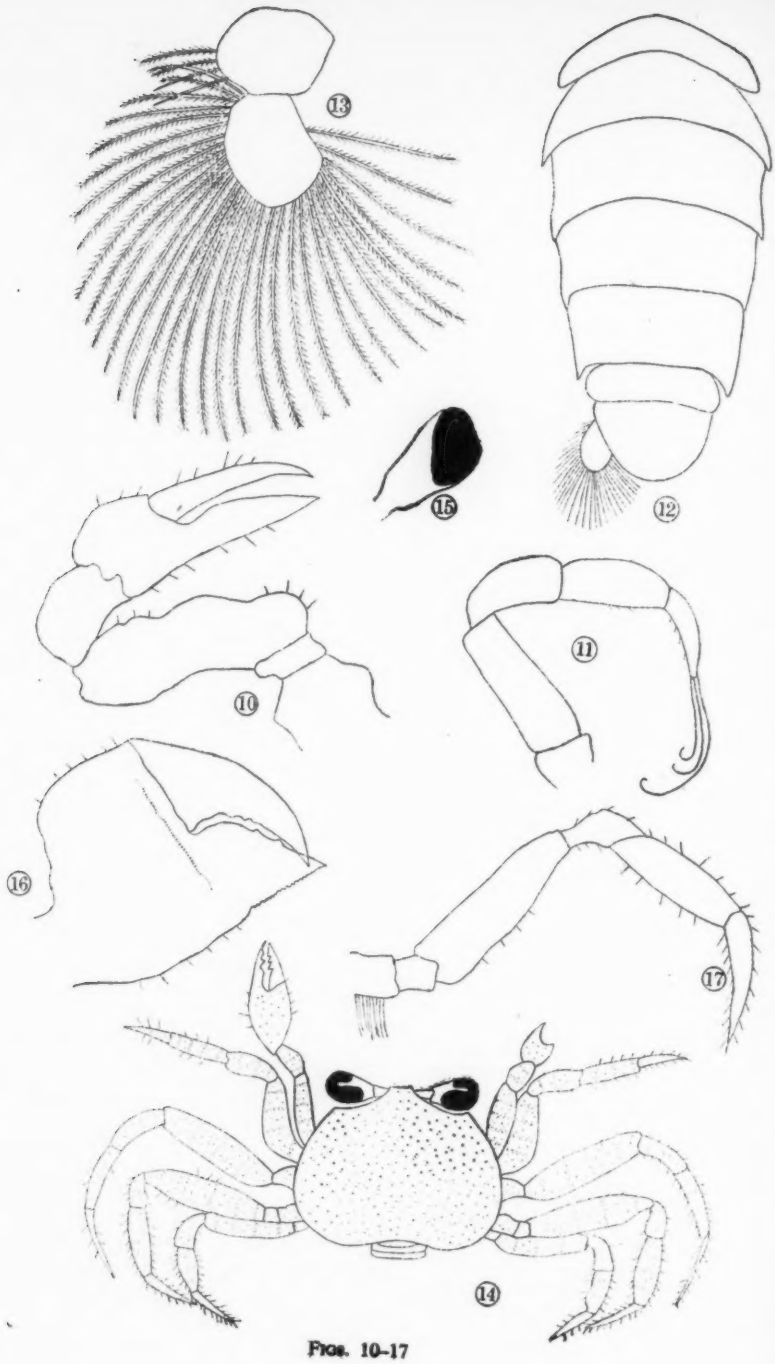
There are a pair of large chelæ (Fig. 10) almost of equal size and four pairs of ambulatory legs. The dactylus of the last leg has three long feelers (Fig. 11).

The abdomen consists of seven segments including the telson which is rounded (Fig. 12). Five pairs of pleopods are present. In the last pair, the exopodite has 32 setæ (Fig. 13).

*The Gill Formula.*—Six gills *plus* three epipodites. The gill of the cheliped is a great gill. The gills are of the same number as in the adult.

*First Crab Stage of* O. platytarsis (Fig. 14)

On 18–12–1951 the megalopa moulted and the first crab emerged. It was quite large and of carapace length 6·3 mm. and breadth 6·8 mm. (measurements taken on 20–12–1951 when it was killed and preserved). Twelve hours after moulting it was able to lead a normal *Ocypoda* life. It dug a number of burrows and remained mostly inside one of the burrows.




FIGS. 10–17

FIGS. 10–17. Fig. 10. Major cheliped of the megalopa of *O. platytarsis*, × 15. Fig. 11. Last ambulatory leg of the megalopa of *O. platytarsis*, × 15. Fig. 12. Abdomen of the megalopa of *O. platytarsis*, × 15. Fig. 13. Last pleopod of the megalopa of *O. platytarsis*, × 50. Fig. 14. First crab stage of *O. platytarsis* (from the cast skin), × 4. Fig. 15. Eye of first crab stage, × 15. Fig. 16. Major cheliped of first crab stage of *O. platytarsis* showing the stridulating ridge consisting of fine tubercles on the palm, ×30. Fig. 17. Second ambulatory leg of the first crab stage of *O. platytarsis* showing tuft of hair at the base, × 15.

(*N.B.*—Figures have been reduced to $^{11}/_{25}$ the magnification given along with the explanation of figures.)

It lived on organic matter from the sand and threw the unwanted sand in the form of pellets. Once when a Mysid was given, it ate the animal most ravishly.

Eyes are jet black, long and stalked but without horns (Fig. 15). The carapace has a mottled grey colour. Chelæ and legs are light brown with dark brown stripes. The carapace has a bulged appearance unlike the flat carapace of the adult. The presence of a stridulating ridge consisting of small tubercles on the inner surface of the major chela is a clear indication of the specific identity of the form (Fig. 16).

A first crab stage was obtained on 20–5–1952 and was reared in the laboratory. This moulted to the second crab stage on 28–5–1952. The crab died on 16–6–1952. At this stage the carapace is not bulging and is more like the condition seen in the adult. The horns of the eyes, however, are not yet developed.

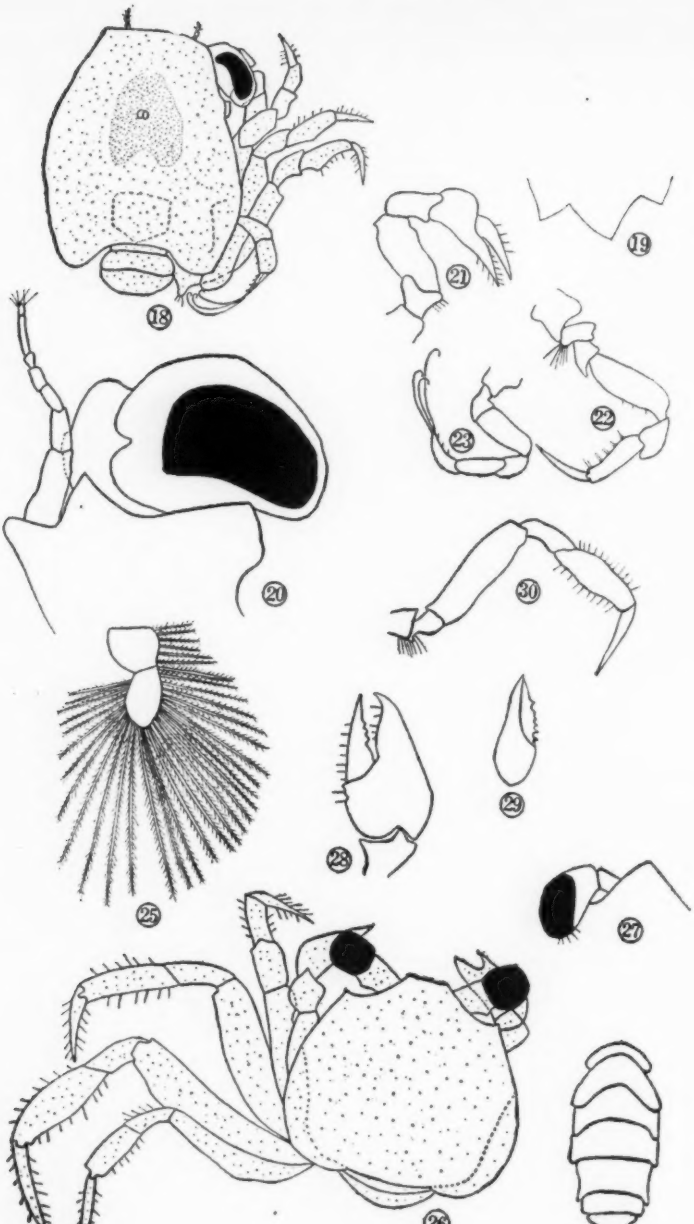
## II. MEGALOPA AND CRAB STAGE OF *Ocypoda cordimana* (Figs. 18–30)

### *Megalopa*

A large number of megalopæ (Fig. 18) of *Ocypoda cordimana* were collected during high tide on the Waltair beach on 30–8–1951. Of these only one lived for about a month and then died without casting off its skin. During April and May 1952 a few more megalopæ were collected. On 21–5–1952 two of these moulted to first crab stage and the others died. On 12–2–1953 a megalopa was collected in a burrow of about six inches depth and 2/16 inch in diameter about 25 feet from sea at low tide.

The megalopa skin is of length 5·7 mm. and breadth 4·8 mm.

*Colouration.*—The megalopæ exhibit two types of colour schemes alternating with one another during this period of their existence. (1) In the centre of the carapace there is a leaf-like patch of brick red splashed with brown and the rest of the carapace is yellowish. The abdomen is of the same colour as the leaf-like patch of carapace. The pereiopods and the ventral side of the cephalothorax are almost colourless with a slight tinge of brown. In the second scheme of colouration the central part of carapace may be oval in




FIGS. 18–30

FIGS. 18–30. Fig. 18. Megalopa of *Ocypoda cordimana*, × 10. Fig. 19. Rostrum of *Ocypoda cordimana* front view, × 10. Fig. 20. Ventral view of the sub-orbital projection on which the cheliped rests—of the megalopa of *O. cordimana*, × 50. Fig. 21. First chela of the megalopa of *Ocypoda cordimana*, × 10. Fig. 22. Third ambulatory leg of megalopa of *O. cordimana*, × 10. Fig. 23. Last leg of megalopa of *O. cordimana* showing the three feelers, × 10. Fig. 24. Abdomen of the megalopa of *O. cordimana*, × 10. Fig. 25. Last pleopod of the megalopa of *O. cordimana* showing the setæ, × 50. Fig. 26. First crab stage of *O. cordimana* (from the cast skin), × 10. Fig. 27. Eye of first crab stage of *O. cordimana*, × 10. Fig. 28. Major chela of first crab stage of *O. cordimana*, × 10. Fig. 29. Minor chela of first crab stage of *O. cordimana*, × 10. Fig. 30. Second ambulatory leg of the first crab stage of *O. cordimana*, × 10.

shape and navy blue in colour and the rest of carapace light blue. The pereiopods are of dark brown shade and the ventral surface of the megalopa is colourless. The two colour patterns have been observed to alternate in the same form at intervals of about 48 hours or they may be constant. The colour changes go on for some time till just prior to ecdysis the leaf-shaped patch of the carapace becomes brilliant orange and the rest of the carapace has a cream colour.

The megalopæ which were reared in finger bowls spent most of the time swimming and all of a sudden they went to the bottom and burrowed into the sand with a characteristic cork-screw-like movement. When a megalopa swimming about actively is taken in the hand, the animal folded its legs tightly close to its body and remained motionless for some time. Later it tried to run, tucking its abdomen underneath the sternum.

The megalopæ thrived well in the laboratory on pieces of the mole crab, crab muscles and mantle pieces of bivalves. As to their food in nature, examination of the stomach-contents of freshly collected specimens showed occasional presence of such bits of partly digested muscle.

The megalopæ of *O. cordimana* resemble that of *O. platytarsis* except in the shape of the telson and the number of setæ in the last pleopod.

*First Crab Stage* (Fig. 26)

The megalopa cast off its skin on 21–5–1952 and the first crab stage of *O. cordimana* emerged. It has a maximum length of 5 mm. and a maximum breadth of 5 mm. The carapace is grey with patches of black. The eyes are jet black and the rest of the body is grey. After five days a few pink chromatophores appeared on the carapace and appendages. It was very active and took in quantities of sand apparently for the organic matter contained in it and discarded the sand in the form of pellets by the side of the burrow.

## KEY TO THE MEGALOPÆ AND FIRST CRAB STAGE OF *O. platytarsis* AND *O. cordimana*

*Megalopæ*

The megalopæ are quite large in size with thick cuticle. Special grooves are present at the sides of the body for folding their appendages tightly close to their bodies. Between the second and third ambulatory legs there is a tuft of hair. The last joint of last leg is provided with three long feelers.

Carapace length ranges from 6·8 to 7·0 mm. and breadth from 6·1 to 6·3 mm. Just before moulting to first crab stage the carapace is greyish. Telson rounded and exopodite of the last pleopod with 32 setæ . . . . . *O. platytarsis.*

Carapace length ranges from 5·3 to 5·8 mm. and breadth from 4·26 to 4·8 mm. and just before moulting to first crab stage, a bright orange leaf-shaped marking is seen in the centre of carapace. Telson is triangular with rounded apex. The exopodite of the last pleopod with 30 setæ . . . . . *O. cordimana.*

*First Crab Stages*

Crabs with stalked eyes but without horns. (Horns make their appearance in later stages of development of only *O. platytarsis.*) Tuft of hair between second and third ambulatory legs.

Stridulating ridge of tubercles present on the inner side of major chela . . . . . *O. platytarsis.*

Stridulating ridge absent . . . . . *O. cordimana.*

## DISCUSSION

Say (1817) remarks that *Monolepis inermis* which has subsequently been identified as the megalopa of *Ocypoda albicans* (Smith, 1880), lies buried just below the surface layer of sand and that its presence is evident by small mounds of sand spread over it by the action of waves. He further explains that they wait in this way for the return of the tide. Smith (1880) rightly contradicts Say's observation stating that the megalopæ " were more likely waiting the final change to the terrestrial stage". He lays stress on the presence of the tuft of hair between the bases of second and third pair of ambulatory legs, a character of the adult, connected with respiration and observes that this ' fits the animal for its terrestrial existence as soon as it is thrown upon the shore'. Crane (1940) corroborates Smith's observation of the buried stage of the megalopæ on the beach as the stage prior to the

moulting into the first crab stage. As additional evidences for this view she states 'the outstanding characteristics of the megalops of *Ocypoda* are its obesity, the thickness of its cuticle and its provision for folding the appendages tightly against the body'. She correlates the structure of the *Ocypoda* megalopæ with their habits. Her observation is well exemplified by the megalopæ of *Ocypoda platytarsis* and *Ocypoda cordimana*. In addition to having all the peculiar structures mentioned by Crane, I observed that the number and structure of gills are similar to those of the adult and it is needless to say that these peculiar structures help the megalopa to tide over the adverse circumstances when it lands from waves on shore. The megalopæ were caught by the author while they were in the act of burrowing or just burrowing into moist loose sand during high tide. In the laboratory the megalopæ were observed to disappear into the sand by burrowing in a cork-screw-like movement. One of the megalopæ lived in the laboratory for a month and died without moulting. During high tide, I observed some first crab-stages of these two species running towards land. On 12–2–1953 and subsequently megalopæ were collected in burrows of six inches depth and 3/16 inches diameter about 25 feet or more from the sea during low tide. This removes all doubt regarding their life on land. The megalopæ burrow in sand, lead a semi-terrestrial existence for some days before moulting to first crab stage.

Crane collected megalopæ of *O. occidentalis* further away from the habitat of *O. gaudichaudi*; these show difference in the number of setæ of the last pleopod and patterns of the sternal sculputre. Her identification is also based on how near the megalopæ were collected with reference to the habitat of the adult species. Such identification cannot be final for it is quite possible that the megalopæ of a particular species can be carried by the action of currents and waves to places nearer to the habitat of some other species. I find that the megalopæ of *O. platytarsis* and *O. cordimana* are very similar to one another in many respects except for size, colouration, number of setæ on the last pleopod and the shape of the telson. It was possible to identify the species correctly only after getting the first crab stages.

The megalopæ of the Indian forms differ only slightly from those of the American forms; the setæ of the last pleopod of *O. platytarsis* and *O. cordimana* are 32 and 30 (Fig. 25) respectively whereas in *O. occidentalis*, *O. albicans* and *O. gaudichaudi* the number of setæ are 22, 23 and 26 respectively. The telson of the American forms are round as in *O. platytarsis*, while the telson of *O. cordimana* is triangular with a blunt apex (Fig. 24).

The first crab stage of *O. platytarsis* differs from the adult stage in the following respects.

1. The carapace is strikingly bulging, so different from that of the adult.

2. The eyes though stalked are without horns.

3. In the manner of taking food, it differs from the adult but is similar to *O. macrocera.* It obtains its nutrition by feeding on detritus found in sand; a small quantity of sand is taken into the south and after taking in the fine particles of food contained therein, the sand is thrown out close to the burrow in the form of pellets of uniform size and shape.

The first crab stage of *O. cordimana* differs from the adult stage in the following respects:

1. The carapace is quite bulging though to the same extent not as in the first crab stage of *O. platytarsis.*

2. In the manner of taking food it behaves similar to *O. macrocera.*

## SUMMARY

1. The megalopæ of *O. platytarsis* and *O. cordimana* were for the first time successfully reared in the laboratory to the first crab stage.

2. The megalopæ of the two species differ in size, colour, shape of telson and number of setæ on the last pleopod.

3. The megalopæ of both species burrow in the sand of the intertidal zone before metamorphosing into the first crab stage.

4. A key for the identification of the megalopæ and the first crab stages of the two species is given.

## ACKNOWLEDGMENT

My thanks are due to Dr. P. N. Ganapati, M.A., D.Sc., Head of the Department of Zoology, for his valuable help and encouragement during the course of this work and also for kindly going through the manuscript and offering valuable suggestions. My thanks are also due to the Government of India for the award of a Research Fellowship during the tenure of which the work was undertaken and completed and to the Andhra University for affording me all facilities for the work.

## BIBLIOGRAPHY


1. Crane, J. .. " On the post-embryonic development of Brachyurous crabs of the genus *Ocypoda*, " *Zoologica, N.Y.*, 1940, **15**, 65–82, 8 figs.
2. Dana, J. D. .. " * United States Exploring Expedition, " *Crustacea*, 1852, **13**, 2 vols. and folio atlas of 96 plates.
3. Gurney, R. .. " Larvæ of Decapod Crustacea, " Ray Soc., 1942, London.
4. Hyman, O. W. .. " On the development of Gelasimus after hatching, " *Jour. Morph.*, 1920, **13**, 485–524, 12 pls.
5. Lebour, M. V. .. " The larval stages of the Plymouth Brachyura, " *Proc. Zool. Soc.*, London, 1928 *b*, 473–560, 16 pls.
6. ———— .. " * Larval Crustacea (Decapoda and Stomatopoda) Expedition S.A.P. Prince Leopold of Belgium, Duke of Brabant to the extreme east, " *Bull. Mus. Hist. nat. de Belg.*, 1934, **10**, No. 8, 24, 19 figs.
7. Menon, M. K. .. " The-life histories of Decapod Crustacea from Madras," *Bull. Madras Govt. Mus. N.S., Nat. Hist. Sect.*, 1933, **3**, 1–145, 10 pls.
8. ———— .. " Decapod Larvæ from the Madras Plankton, " *Ibid.*, 1937, **3**, 56, No. 5, 9 pls.
9. Rathbun, M. J. . " * Brachyuran crabs collected by the Williams Galapagos Expedition, 1923, " *Zoologica, N.Y.*, 1924, **5**, 153–59, 1 pl. 1 texte-fig.
10. Say, T. .. " An account of the Crustacea of the United States, " *J. Acad. Nat. Sci., Philad.*, 1817, **1**, 155–69.
11. Smith, S. I. .. " The megalops stage of *Ocypoda*, " *Amer. J. Sci.*, 1873 *c*, 6 (3), 67, 69.
12. ———— .. " Occasional occurrence of tropical and sub-tropical species of Decapod Crustacea on the coast of New England, " *Trans. Conn. Acad. Arts Sci.*, 1880 *b*, **4**, 254–67.


* Not referred to in the original.

# PATHOLOGICAL STUDIES OF *GLŒOSPORIUM PSIDII* CAUSING DIE-BACK OF GUAVAS

By R. N. Tandon and R. K. Agarwala
(*Department of Botany, University of Allahabad*)

Received February 3, 1954
(Communicated by Prof. Shri Ranjan, F.A.SC.)

## Introduction

Allahabad has the proud privilege of being the best guava growing centre in the state. For the last few years a very high death rate of branches and wilting of guava plants has been observed in certain orchards at Allahabad. It is fast becoming a menace to orchard owners. The trouble is not confined to Allahabad but has been observed at other places also.

A thorough survey of Allahabad guava orchards was undertaken and severe losses were observed during the rainy season when the growth of the new twigs was considerably checked due to this trouble. Hardly any plant was free from this disease.

Die-back is a common disease on many plants. Waterston (1941) reported *Glœosporium psidii*, the cause of 'leaf spot' disease of guava. There is no other record of the Die-back organism of guava.

It has been shown by Baker and Wardlaw (1937) and Baker (1938) that certain fungi may remain inactive in the tissues of the host for considerable time before the formation of lesions gives a visible clue of their presence. This type of infection was termed by them as 'Latent Infection'. It was further demonstrated that the infections occur early in the development of fruit and that they were confined to the upper layers of the rind irrespective of the age of the fruit at the time of infection. The lesions become visible only when a certain stage of ripening has been reached. Simmonds (1941) has described latent infection associated with species of *Glœosporium* and *Colletotrichum* in immature banana fruits infected with *Glœosporium musarum*. It remained in latent state for about 5¼ months after which the characteristic anthracnose lesions developed and the fungus became active.

There has been practically no work on *Glœosporium psidii* isolated from guavas. It was, therefore, decided to undertake a detailed physiological and pathological study of *Glœosporium* isolated from the stems of guava trees.

## Material and Method

The material for isolation was collected from the trees of *Psidium gujava*. The methods used for isolating the fungus were the same as described by Tandon (1952). It invariably gave *Glœosporium psidii*. Single spore cultures were prepared by dilution method and they formed the parent cultures.

In order to test the pathogenicity of the isolates a large number of artificial inoculations were carried out on the injured and uninjured plants. The stem and leaf inoculations were made on the new young growth near the top of a twig, or at the growing point as well as at older regions of the plants. To avoid infection and drying of the spores, moist cotton pads were placed on them. In case of fruits, inoculations were made by the method described by Granger and Horne (1924). They were also inoculated through injured and uninjured regions by placing the inoculum at calyx and stem ends and the injured surface of the fruits as described by Tandon (1948). Controls were maintained in each case.

## Symptoms

The most characteristic symptom of the disease is that the plant begins to die backwards from the top of a branch. Young shoots, leaves and fruits, while they are still tender, are readily attacked. The greenish colour of the growing tip is changed into dark brown colour which later on develops into black necrotic areas extending backwards and causing the die-back. The disease becomes more noticeable after a period of incubation in the infected buds and twigs. The brown spots, formed previously, change into silvery grey and ultimately develop a pronounced demarcating ring or girdle at the junction of the diseased and healthy part. This ring may either be very distinct or it may gradually diffuse into healthy green part of the shoot.

The fungus makes its track from the infected twigs to the petioles and ultimately the young leaves are attacked. In severe cases of infection they are entirely killed but ordinarily they may have dead areas on the margins or tips with distortion of the remaining portions. These may droop down or fall on account of a slight jerk of the gentle breeze. The dried twigs altogether devoid of leaves are ultimately left (Plate II, Fig. 1).

In moist weather when disease is more vigorous acervuli of the fungus may be seen as black dots scattered throughout the dead parts of the twigs, and if they are kept in moist chambers they ooze out the pinkish masses of spores.

## OCCURRENCE AND EXTENT OF DAMAGE

All sizes of trees from those located in the nursery beds to the old trees growing in the orchard are subjected to attack. In Minto Park, Allahabad (Govt. Garden), the guava orchards were severely infected in 1952. Several trees died out completely without bearing a single fruit while others were nearly half dead (Plate II, Fig. 2). The ripened fruits hanging in these infected trees were clearly showing the pinkish masses of acervuli causing the rot on the outer skin of the fruit.

A regular fortnightly survey was carried out for one year (June 1952 to July 1953) in different orchards at Allahabad and the amount of damage during different periods was carefully recorded.

In June when there was slight rain, only the old twigs showed the symptoms of die-back. New shoots with tender stems and leaves were appearing and they showed no apparent symptom of any disease. In July it was often cloudy, the temperature was lower and the atmosphere was very humid. The growth of the new shoots was vigorous and no evident symptom of the disease could be noticed in young shoots. In the first fortnight of August, the disease caused the death of small tender growing twigs. From August to October, the infected twigs withered and shrivelled at their tips upto a distance of one to several inches. This greatly reduced the younger growth and prevented blooming in severe cases. The disease appears in epidemic form from August to September only and under favourable conditions it may reach the main trunk. If due to any reason the infection does not prove very severe only the growth of the first sprout is checked, while other subsequent sprouts do not show the sign of arrested growth of the plant. When the infection reaches a node or some point of bifurcation of the two twigs, it again shows the sign of dormancy for a short or even for a fairly long period.

The die-back of guava trees becomes more prominent with the advent of time. It can also be noticed in December and later months after which the older leaves and twigs remain immune from further attack. Whenever the weather conditions are almost on the two extremes and the atmosphere is less humid (*viz.*, from January to June) the further progress of the disease is greatly inhibited. There are no new tender shoots and leaves on the trees for the parasite to attack. The older ones escape damage as these are sufficiently hard and woody. The percentage infection in the months of July, August, September, October, November and December was 5, 13, 25, 17, 8 and 4 respectively.

Observations confirmed that hot and dry weather of April to June, and cold season of January to March prevents the organism from starting new infection during these months.

## Pathogenicity

Pathogenicity tests on leaves, twigs and fruits were conducted both in the laboratory and garden during the rainy season as well as in the summer months.

*Twigs and Stalks.*—During the rainy season the inoculated twigs began to change their greenish colour to brown after 15–20 days. The intensity of infection continued to increase and the young twigs exhibited dry conditions upto a length of 1–1½ inches. It was further noticed that the fungus placed inside the injured areas succeeded in infecting the twigs while the inoculations made without injury gave negative results. In summer even injured regions failed to show any infection without application of moist cotton pads and even under such conditions the percentage infection was low. The necessity of applying the cotton pads was not felt in the rainy season when the infection extends towards the trunk more rapidly than in summer months. The twigs which were inoculated soon after the drizzling or when the rain started and continued for a week or more showed withering within a week. The damage was more severe, if the clouds were present. Under such conditions the new growth of the current season was largely attacked. It, therefore, confirmed that humidity played an important part in the growth of the organism. Successful re-isolations of *Glœosporium psidii* were made from most of the natural or artificially infected parts. The old branches which were sufficiently hard and woody resisted the entrance of the casual organism as the old stalks of the trees did not develop any infection.

*Leaf Infection.*—The inoculations made on the upper and the lower surfaces of the leaves showed the symptoms of the disease which appeared after one week on the lower surface of the leaf only. The young leaves which were infected showed dead areas on the margins and the tips with distortion of the remaining portions. The speed of the disease from tip to the base of the leaf was sufficiently rapid in some cases. Inoculations on older leaves invariably failed to develop any disease.

*Flower Infection.*—Small flowers of guavas and their opened buds were sprayed with the atomizer containing a suspension of the spores of *Glœosporium psidii*. Unopened buds showed infection within two or three days and failed to develop further. The petals turned brown and the buds

fell off without opening. Flowers also showed the same symptoms and they ultimately dropped off before fertilization.

Attempts were also made to observe the effects of a drop of spore suspension on the stigmatic surface of each pistil. The flowers selected for this purpose were the unopened buds which were carefully opened with the help of fine needles. Few out of these opened buds were not inoculated and were used as controls; while the spore suspension was placed with the help of sterilized eye dropper on the stigmatic surface of others. It was observed that the stigmas inoculated with spore suspension developed dark brown colour after 24 hours and in many cases the entire flower including the stalk dropped off within 3–4 days. The infected ovary was occasionally left hanging on the tree and subsequently it dried into dark brown colour and fell on the ground after a few weeks. In still fewer cases, the fertilized infected ovary persisted to grow into a fruit which ultimately changed into a dry hard stony structure with dark brown or black brown colour (Plate II, Fig. 3). These were developed due to infection at early stages. The unsprayed flowers (Control) developed normal fruits.

*Fruit Infection.*—Mere contact of the diseased and the healthy fruit was sufficient to develop a rot in ripened fruits but it never developed into a mummy. The rot is usually slow unless the fruit is ripe. At all subsequent stages, the organism could cause a rot of mature fruit near the point of infection. Pink to dark brown circular patches of acervuli were produced on the rotten areas. These small patches increased in diameter and they covered a large surface of the fruit within two days after which cottony mycelium appeared at the surface of the fruit provided they were kept in moist chambers. It was, however, noticed that if the infected fruits were allowed to remain on the tree or in the laboratory they were usually deformed and they became worthless as they gradually became hard dry and shrunken.

Besides artificially developed hard and stony fruits other small dried blackish brown or sometimes black fruits of guavas (commonly known as mummies) were frequently observed hanging on the diseased guava plants. An attempt was made to find out whether they were developed, as a result of infection of flowers or of fruits at different stages of their development. For this purpose guava fruits of different ages were inoculated on the tree in their natural condition. The inoculations were started soon after the fertilized ovary became rounded. In one set of experiments the raw fruits of approximately one inch in diameter were inoculated by Granger and Horne's method (1924). In another set only the spore suspension was sprayed with the atomizer on the fruit of similar size. At this stage the young

fruits were as hard as stone and were rich green in colour. It was observed that all fruits on which spores were sprayed showed no infection under any condition at any stage. The fruits which were inoculated by Granger and Horne's method also did not show any trouble in early stages but infection was observed when those fruits reached maturity. The organism survived in latent stage inside the rind for about 2½–3 months. When the fruits ripened it resumed its activity to produce typical rot on the outer surface of the fruit. It was also observed that the inoculum placed in the subcuticular region of injured raw guavas developed a mass of hyphæ which remained as such till the fruits ripened. This indicated that during the latent period the fungus survived in the form of hyphal structure. Numerous appressoria were formed near the subcuticular surface.

The absence of active parasitism in the green guava fruits even though the fungus grows on its extract indicates that the hard tissue prevents the fungus from growing. It remains dormant till the tissues become quite soft. It was noticed that when the organism was grown on guava extract medium, it sporulated more or less with the same rapidity as on the ripened guava extract medium; while in inoculation experiments, the fungus did not develop any symptom of disease till the fruits were fully matured. It is very likely that due to very hard nature of the host (unripe guava) the fungus finds it difficult to develop on it. Blackman and Welsford (1916) showed that the rupture of the cuticle and subsequent penetration was due to mechanical pressure exerted by the penetration hyphæ. Dey (1933) also reported mechanical penetration in the infection of beans by *Colletotrichum lindemuthianum* as well as on lemon leaves infected by *Colletotrichum glœosporioides*. The pressure needed for penetrating raw guavas does not develop in the present case but on ripening the cellular mass is loosened and hence the intercellular development of the organism becomes possible. The rapid growth of the parasite starts after a long dormant period. The spores penetrate through the soft cuticle with the help of fine infection thread which afterwards develops a thick mass of mycelium adjacent to the epidermal cells and the characteristic acervuli are produced on the outer skin.

## Cross Inoculations

Cross inoculations of *Glœosporium* isolated from *Citrus aurantifolia*, *Citrus Karna*, *Citrus medica* and *Psidium gujava* were tried and it was found that *Glœosporium psidii* could effect guava plants alone and other organisms failed to infect except the host from which they were isolated.

## CONTROL

The disease was most active when the humidity was high and new branches appeared. It was also more serious on trees growing close together or in shade.

An attempt was, therefore, made to control the die-back or the development of mummies. Some proprietory chemicals, *viz.*, Zerlate, Parazate, Diathene Z-78 were tried but none of them could check the disease.

Finely powdered sulphur, lime sulphur 15%, 25%, peronox ·22%, ·33% and bordeaux mixture 2:2:50, 3:3:50 and 5:5:50 were applied either on the diseased plants directly or after removing the dead twigs. The spraying or dusting was carried out after the rains and three applications were given at an interval of three weeks each. It was observed that they were successful in reducing the losses. The application of fungicides was more effective when it was applied after removing the dead branches.

Out of all these fungicides used, bordeaux mixture 3:3:50 was more effective and was closely followed by peronox. The percentage losses on lime sulphur 15%, 25%, sulphur, peronox ·22%, ·33%, and bordeaux mixture 2:2:50, 3:3:50, 5:5:50 were 50, 50, 28·5, 10, 12, 12·5, 6·6, and 13·5 respectively. The losses were about 15% higher when dead twigs were not removed.

## SUMMARY

1. *Glœosporium psidii* was isolated from die-back of guava trees. A detailed study of the symptoms, the amount and extent of damage has been recorded. It was found that severity of the disease increased from middle of August to beginning of October.

2. The pathogenicity of the organism was established and it has been found that it can readily attack the young tender shoots, injured leaves, flowers and buds. It failed to attack mature twigs and leaves.

3. It has been established that mummies are produced if buds and flowers are infected at an early stage.

4. The fungus can live in dormant condition for about 2½–3 months. It subsequently resumes its activity and develops the rot when the fruits ripen.

5. None of the fungicides so far tried could control the disease fully but the application of 3:3:50 bordeaux mixture and ·22% or ·33% peronox gave encouraging results.

FIG. 1

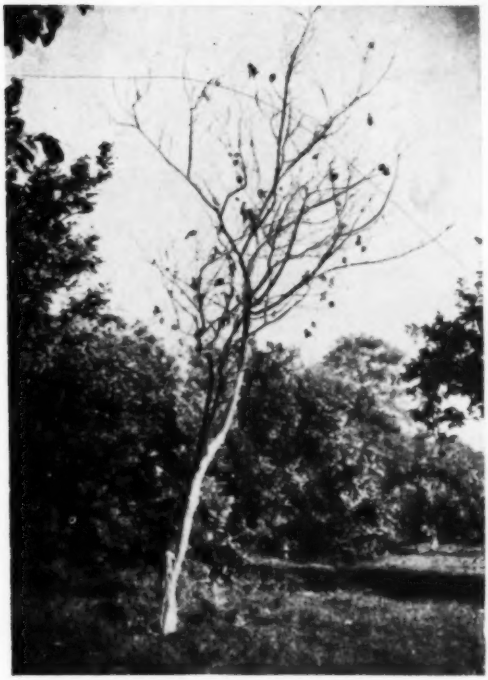


FIG. 2

FIG. 3

FIG. 4

## References


Baker, R. E. D. .. "Notes on the control of mango anthracnose," *Trop. Agric.*, XV, 1938, **1**, 12–14.

——— and Wardlaw, C. W. .. "Studies in the pathogenicity of tropical fungi," *Ann. Bot. n.s.*, 1937, **1**, No. 1, 59–66.

Blackman, V. H. and Welsford, E. J. "Studies in the physiology of parasitism," *Ann. Bot.*, 1916, **30**, 389–98.

Dey, P. K. .. "Studies in the physiology of appressorium of *C. glæosporiodes*," *Ibid.*, 1933, **47**, 305–12.

Granger, K. and Horne, A. S. .. "*Ibid.*, 1924, **38**, 212–15.

Simmonds, J. H. .. "Latent infection in tropical fruits discussed in relation to the part played by Sps. of *Glæosporium* and *Colletotrichum*," *Proc. Roy. Soc., Qd.*, 1941, Iii, **10**, 92–180.

Tandon, R. N., Babu Singh and Grewal, J. S. .. "Leaf spot disease of 'Litche'," *Proc. Nat. Acad. Sci.*, Sec. B, Parts I–V, 1952, 21–28.

——— and Tandon, M. P. .. "Pathological studies of *Pestalotia* Sp.," *Ibid.*, Sec. B, Part I, **17**, 1948.

Waterston, J. M. .. "Leaf spot disease of Guava (*G. psidii*)," *Rep. Dep. Agric. Bermuda*, 1941, 6–8.


## Explanation of Plate

Fig. 1. Symptoms incited by *G. psidii* on guava stems.

Fig. 2. Showing severe infection due to which the plant is killed. A few mummies are seen.

Fig 3. Showing the infected ovary which developed into a mummy.

Fig. 4. Showing ripened fruits (towards left) inoculated which *Glæosporium psidii* and a healthy fruit used as a control (towards right).

# DESOXYRIBONUCLEIC ACID (DNA) CONTENT IN THE FEMALE GAMETOPHYTE AND NUCELLUS OF RHOEO DISCOLOR

By M. Nagaraj, M.Sc. (Chicago), Ph.D. (Chicago)
*(Department of Botany, Central College, Bangalore)*

Received August 24, 1954
(Communicated by Principal Dr. B. S. Madhava Rao, F.A.SC.)

## Introduction

With the development of the view that the Desoxyribonucleic acid (DNA) content of an animal or a plant cell remains remarkably constant, little affected by changing cell states (Boivin, Vendrely and Vendrely, 1948; Vendrely and Vendrely, 1948, 1949, 1950; Mirsky and Ris, 1949; Ris and Mirsky, 1949) attention has been directed towards determining the DNA content of tissue cells in different organisms with a view to establishing its relationship with the degree of ploidy. Largely due to recent studies (Swift, 1950; Ogur *et al.*, 1951; 1952) it has become clear in plants that corresponding to the number of chromosome sets in the nucleus the nuclear DNA also exhibits a strict variation. A recent analysis (Nagaraj, 1954) of the DNA content of the female gametophyte and nucellar cell nuclei in *Tradescantia paludosa* emphasised this relationship, the haploid gametophyte nuclei showing a DNA content half of that found in the surrounding nucellar cell nuclei. It was felt that an extension of these studies would be valuable. *Rhoeo discolor* (Commelinaceæ) was chosen.

## Materials, Methods and Results

This work was done during the author's stay at the Department of Botany, University of Chicago, Chicago, U.S.A. The material came from the green-houses of the Department of Botany. Young flower buds and individual ovaries were fixed in neutral formalin immediately after removal from the plant. Conant's modification of Navaschin's solution was also used as a fixative; both fixatives gave satisfactory results. Paraffin sections 16–30 $\mu$ thick, were cut and stained with the Feulgen reagent. The amount of Feulgen dye in individual nuclei of the 2-nucleate, 4-nucleate and 8-nucleate female gametophyte as well as the nuclei of the nucellar cells was estimated by photometric methods. The entire work was done in Dr. H. Swift's laboratory, University of Chicago, during 1952.

The details of the apparatus were as follows: Monochromatic light was isolated with a Beckman Spectrophotometer with a slit width of 0·1 or 0·3 mm. The microscope was a Leitz model with achromatic-aplanatic condenser, N.A.; 1·4, 90 X Leitz 2-mm. oil immersion apochromatic objective, N.A.; 1·32 and 20 X Bausch and Lomb coated hyperplane ocular containing an iris diaphragm, to minimize distortion from internal reflection. The microscope image, enlarged 1,000 times was projected on a field diaphragm which allowed an area 2, 3, 4 or 5 mm. in diameter taken in the center of the nuclear image to fall on the phototube. Measurements were taken with a battery-powered electron multiplier phototube No. 1 P 21, with output connected with a Farrand Type B control unit and a Rubicon galvanometer. Measurements were made at the absorption peak of the Feulgen dye, 615 m$\mu$.

The methods of making measurements were as follows:—

(*a*) The nucleus to be measured was moved into the optical axis of the system and centered in the iris diaphragm opening. (*b*) The aperture was then reduced until it just enclosed an area in the nucleus, 3 $\mu$ or 4 $\mu$ in diameter. (*c*) The phototube was then moved into place and the galvanometer deflection was recorded ($I_n$). There is a large nucleolus in the nucleus of this species and in all cases, it was excluded from the region of the nucleus measured. (*d*) The nucleus was then moved out of the field, a clear area was brought into position, and maintaining the same aperture, the background intensity was recorded ($I_0$). The initial reading ($I_n$) divided by the background reading ($I_0$) gives the transmission (T): $T = I_n/I_0$. The extinction (E) was then calculated $\left(E = \text{Log } \frac{1}{T}\right)$. The extinction is the value which varies directly with concentration and thickness of absorbing material according to Beer-Lambert Law. The volume of the cylindrical region cut from a spherical nucleus is given by $V = \frac{4}{3} \pi (R^3 - H^3)$ where R is the radius of the sphere and H is half the height of the cylinder. For each nucleus measured R and $r$ were determined ($r$ is the radius of the cylinder) and the extinction was divided by the fraction (F) of the total nuclear volume indicated in the cylinder, where

$$F = \frac{R^3 - (R^2 - r^2)\, 3/2}{R^3}.$$

For convenience of calculation the area of the measured plug ($\pi r^2$) was substituted by the proportional square of the plug radius ($r^2$) and the final calculation of the total amount of the absorbing substance, in arbitrary units

per nucleus, becomes $\frac{Er^2}{F}$. The majority of the nuclei measured were spherical. In the case of a few which were probably spheroids, the longer and shorter axes were averaged and the mean was taken as the radius of the sphere.

Ovules of *Rhoeo discolor* are crassinucellate, bitegmic and orthotropous. One of the cells in the nucellus enlarges itself to become the megaspore mother cell, which is diploid and which undergoes two divisions to form a tetrad of megaspores which are haploid. Usually the chalazal megaspore develops further; the others degenerate. The functional megaspores undergoes three successive divisions resulting in the formation of 8-nucleate female gametophyte. The mature gametophyte consists of an egg apparatus (two

TABLE I

*Desoxyribonucleic acid determination of haploid nuclei of female gametophyte of* Rhoeo discolor. *Extinction at* 615 *mμ of the Feulgen reaction of a cylindrical plug in the nucleus of* 3 *μ diameter*

| Diameter of the nucleus in $\mu$ | Galvanometer deflection through the nucleus | Galvanometer deflection through the clear area | Transmission | Extinction | Relative amount |
|---|---|---|---|---|---|
| | $I_n$ | $I_o$ | T | E | $\frac{Er^2}{F}$ |
| 4·4 | 10·8 | 36·6 | 30·0 | 0·528 | 17·7 |
| 5·0 | 11·2 | 28·0 | 40·0 | 0·398 | 17·4 |
| 6·6 | 43·2 | 68·6 | 63·0 | 0·201 | 17·4 |
| 5·4 | 26·0 | 52·0 | 50·0 | 0·301 | 16·0 |
| 5·0 | 11·0 | 25·0 | 44·0 | 0·357 | 15·4 |
| 6·0 | 24·4 | 41·2 | 59·0 | 0·229 | 14·9 |
| 6·0 | 31·4 | 53·6 | 58·5 | 0·229 | 14·9 |
| 5·8 | 10·6 | 18·6 | 57·0 | 0·244 | 14·7 |
| 5·5 | 33·0 | 53·2 | 62·0 | 0·208 | 13·8 |
| 6·0 | 41·4 | 62·8 | 65·9 | 0·180 | 13·6 |
| 6·0 | 29·2 | 47·0 | 62·0 | 0·208 | 13·3 |
| 5·1 | 11·0 | 20·8 | 53·0 | 0·277 | 13·2 |
| 4·8 | 4·7 | 10·0 | 47·0 | 0·328 | 13·1 |
| 4·5 | 8·9 | 19·0 | 47·0 | 0·328 | 13·0 |
| 4·6 | 14·4 | 32·2 | 45·0 | 0·347 | 12·9 |
| 4·8 | 27·0 | 54·2 | 50·0 | 0·301 | 12·8 |
| 5·6 | 34·0 | 57·0 | 59·6 | 0·222 | 12·6 |

TABLE II

*Desoxyribonucleic acid determination of diploid nuclei of nucellus of* Rhoeo discolor. *Extinction at* 615 *mμ of the Feulgen reaction of a cylindrical plug in the nucleus of* 3 *μ diameter*

| Diameter of the nucleus in $\mu$ | Galvanometer deflection through the nucleus | Galvanometer deflection through the clear area | Transmission | Extinction | Relative amount |
|---|---|---|---|---|---|
| | $I_n$ | $I_o$ | T | E | $\frac{Er^2}{F}$ |
| 5·5 | 26·4 | 98·2 | 26·9 | 0·569 | 33·7 |
| 5·2 | 30·0 | 77·4 | 38·8 | 0·409 | 22·8 |
| 5·0 | 12·3 | 43·4 | 26·0 | 0·585 | 29·6 |
| 4·4 | 4·0 | 19·2 | 20·8 | 0·678 | 25·1 |
| 4·4 | 5·8 | 27·0 | 21·5 | 0·658 | 24·6 |
| 4·2 | 4·4 | 25·0 | 17·6 | 0·745 | 25·9 |
| 6·3 | 43·0 | 92·5 | 46·5 | 0·328 | 26·9 |
| 6·3 | 20·0 | 46·0 | 43·5 | 0·357 | 25·8 |
| 7·5 | 36·5 | 69·0 | 52·9 | 0·277 | 29·4 |
| 7·5 | 16·8 | 39·7 | 42·3 | 0·377 | 36·0 |

TABLE III

*Desoxyribonucleic acid determination of fusion nuclei (diploid) of the female gametophyte of* Rhoeo discolor. *Extinction at* 615 *mμ of the Feulgen reaction of a cylindrical plug in the nucleus of* 4 *μ diameter*

| Diameter of the nucleus in $\mu$ | Galvanometer deflection through the nucleus | Galvanometer deflection through the clear area | Transmission | Extinction | Relative amount |
|---|---|---|---|---|---|
| | $I_n$ | $I_o$ | T | E | $\frac{Er^2}{F}$ |
| 7·4 | 24·6 | 58·4 | 42·2 | 0·377 | 34·9 |
| 8·8 | 32·0 | 58·8 | 54·4 | 0·268 | 31·4 |
| 7·5 | 30·2 | 64·8 | 46·6 | 0·328 | 29·0 |
| 7·0 | 29·2 | 60·8 | 48·0 | 0·319 | 27·3 |
| 7·3 | 31·0 | 56·4 | 55·0 | 0·260 | 23·5 |

synergids and an egg cell) a fusion nucleus (formed by the fusion of two polar nuclei) and three antipodal cells. The egg apparatus is situated towards the micropylar end, and the antipodals towards the chalazal end. The fusion nucleus usually lies below the egg apparatus and occasionally occupies a central position in the mature gametophyte. Photometric measurements were made of nuclei in three stages. 17 haploid nuclei, 10 nucellar nuclei and 5 fusion nuclei were measured. The results are given above.

An explanation of the values given in the Tables indicates that while the mean DNA content of the haploid nuclei of the female gametophyte is 14·5, that for the diploid nuclei of the nucellar cells is 27·9 and of the fusion nucleus, 29·2. This offers striking evidence of a correspondence between DNA content and ploidy of the nucleus.

## DISCUSSION

Observations made on plants in regard to DNA content in the different tissues have produced rather conflicting results. For instance, Schrader and Leuchtenberger (1949) found no constancy in the amount of DNA in the different tissues of *Tradescantia*, while extensive observations of Swift (1950) on Corn, *Tradescantia paludosa*, *T. virginiana* and *T. canaliculata* show that DNA occurs in well marked units characteristic of the strain or the species. Nuclei with DNA content 2, 4, 8, 16 and 32 times the haploid content occur, presumably indicating the degree of ploidy in each case. Again during mitosis, DNA doubles in quantity in the interphase immediately prior to division and the amount is halved during meiosis and gamete formation. Recent biochemical determinations of DNA by Ogur *et al.* (1951 and 1952) on *Lilium* and yeast nuclei have shown a regular correspondence between DNA amount and degree of ploidy of the nucleus. The author's own studies (Nagaraj, 1954) have substantiated these results in *Tradescantia paludosa*, the diploid nucellar cell nuclei showing about twice the amount of DNA found in the haploid nuclei of the gametophyte. The present study lends additional support to this view.

## SUMMARY

Photometric determinations of Desoxyribonucleic acid in the haploid nuclei of the female gametophyte and diploid nuclei of nucellar cells show that the former have half the amount of DNA found in the latter and appear to establish a regular correspondence between the amount of DNA in the nucleus and its ploidy.

## References


1. Boivin, A., Vendrely, R. and Vendrely, C. — *Compt. Rend. Acad. Sci.*, 1948, **226,** 1061.
2. Mirsky, A. E. and Ris, H. — *Nature*, 1949, **163,** 666.
3. Nagaraj, M. .. — *Curr. Sci.*, 1954, **23,** 300.
4. Ogur, M., Erickson, R. O., Rosen, G., Sax, K. B. and Holden, C. — *Exp. Cell. Res.*, 1951, **2,** 73.
5. Ogur, M., Minckler, S., Lindegren, G. and Lindegren, C. — *Arch. Biochem. Biophys.*, 1952, **40,** 175.
6. Ris, H. and Mirsky, A. E. — *Jour. Gen. Physiol.*, 1949, **33,** 125.
7. Schrader, F. and Leuchtenberger, C. — *Proc. Nat. Acad. Sci.*, 1949, **35,** 464.
8. Swift, H. .. — *Ibid.*, 1950, **36,** 643.
9. Vendrely, R. and Vendrely, C. — *Experientia*, 1948, **4,** 434.
10. ———— .. — *Ibid.*, 1949, **5,** 327.
11. ———— .. — *Compt. Rend. Soc. Biol.*, 1949, **143,** 1386.
12. ———— .. — *Compt. Rend. Acad. Sci.*, 1950, **230,** 671, 780.

# SOIL AMENDMENTS AND PHOSPHATE AVAILABILITY *

By G. P. Gokhale, M. M. Kibe and N. Narayana
*(College of Agriculture, Poona-5)*

Received March 12, 1954
(Communicated by Dr. M. K. Patel, F.A.SC.)

When soils are treated with phosphatic fertilizers, the added phosphates undergo certain changes. A part of the phosphate in the fertilizer is usually in such an inert condition, for instance the fluor apatites, that no amount of cultural practices can release it for the use of plants. Some of the phosphate may get fixed chemically and some may be adsorbed by the soil colloids. Of the various factors which govern the availability of phosphates, the most important are—the nature of the fertilizer used, the soil reaction, the nature of the clay minerals, the nature of the exchange complex, colloid contents, the amount of iron and aluminium oxides and their state of hydration, the organic matter content, the buffer capacity, and the lime reserve of the soil.

The efficiency of the phosphatic fertilisers as judged by the performance of crop, therefore, wholly depends on how one is able to modify the various soil factors so as to harmonize their working towards the release of an adequate supply of phosphorus to the plants. This brings one to the consideration of the use of soil amendments like sulphur, lime, organic matter, etc., which constitutes an important part of the problem of efficient soil management.

Recently, as a part of the " Grow More Food Campaign " the Bombay Department of Agriculture was preparing manure mixtures for food crops, using groundnut cake, sulphate of ammonia, and a phosphatic fertilizer—bone meal or rock phosphate or superphosphate. The question was raised as to which of the three fertilizers would suit a particular soil zone of the Bombay State. As a beginning in that direction the present investigation was undertaken. In this paper the suitability of bone meal, superphosphate and rock phosphate in the soils of Kolaba District as represented by the soil of the Karjat Farm has been investigated. Incidentally the effect of the addition of the soil amendments, *i.e.*, sulphur, molasses, *mohua* flowers (*Bassia latifolia*) and *chavali* (*Vigna catiang*) green manure on the availability of the phosphate has also been studied.

* Paper presented to the Agricultural Sciences Section of the Indian Science Congress, 1954.

## Experimental

The soil used in these investigations was collected at the low lying area of the Rice Breeding Station, Karjat, District Kolaba. The relevant data on the soil are presented in Table I. Texturally, the soil belongs to the class of 'silty loams' by virtue of the preponderance of finer fractions over the sands. Although there is complete absence of calcium carbonate, the

Table I

| Mechanical Composition | Percentage |
|---|---|
| Moisture .. .. .. | 6·25 |
| Carbonates ($CaCO_3$) .. .. | 0·01 |
| *Organic matter .. .. | 2·01 |
| Clay.. .. .. .. | 20·45 |
| Silt .. .. .. | 32·95 |
| Fine sand .. .. .. | 29·65 |
| Coarse sand .. .. .. | 8·68 |
| **Chemical Composition** | **Percentage** |
| Moisture .. .. .. | 6·25 |
| Loss on ignition excluding moisture | 6·97 |
| Sand and insoluble silicates .. | 68·32 |
| Iron oxide ($Fe_2O_3$) .. .. | 10·40 |
| Alumina ($Al_2O_3$) .. .. | 9·70 |
| Lime (CaO) .. .. .. | 0·99 |
| Magnesia (MgO) .. .. | 0·95 |
| Potash ($K_2O$) .. .. | 0·11 |
| Phosphoric acid ($P_2O_5$) .. | 0·08 |
| Total Nitrogen .. .. | 0·087 |
| Total Organic carbon .. .. | 1·24 |
| C/N ratio .. .. .. | 14·13 |
| | **Per cent milli equivalents** |
| Exchangeable Ca .. .. | 26·42 |
| Exchangeable Mg .. .. | 14·64 |
| Exchangeable K .. .. | 0·26 |
| Exchangeable Na .. .. | 2·67 |
| Total Exchangeable bases .. | 43·99 |
| pH value .. .. .. | 6·8 |

* Calculated from Organic carbon determined titrimetrically.

soil contains fair amounts of HCl soluble lime. Compared with the normal run of the soils of the Bombay State, it is fairly well supplied with nitrogen and organic matter. The potash status of the soil is not high and so are the contents of HCl soluble phosphoric acid. In spite of the fact that Karjat receives on an average 130″–140″ precipitation every year, the replaceable bases are not very low and the exchange complex is saturated with divalent bases to the extent of about 90 per cent. As a result, the reaction of the soil is just near neutrality.

Sixteen lots of soil each weighing three pounds were made and distributed over four sets comprising different treatments as shown below:—

1. Soil alone (control).
2. Soil + Superphosphate.
3. Soil + Superphosphate + Sulphur.
4. Soil + Superphosphate + Molasses.
5. Soil + Superphosphate + Mohua flowers.
6. Soil + Superphosphate + Green manure (*Vigna catiang*).
7. Soil + Rock phosphate.
8. Soil + Rock phosphate + Sulphur.
9. Soil + Rock phosphate + Molasses.
10. Soil + Rock phosphate + Mohua flowers.
11. Soil + Rock phosphate + Green manure (*Vigna catiang*).
12. Soil + Bone meal.
13. Soil + Bone meal + Sulphur.
14. Soil + Bone meal + Molasses.
15. Soil + Bone meal + Mohua flowers.
16. Soil + Bone meal + Green manure (*Vigna catiang*).

The requisite quantities of fertilizers calculated to supply 1 gm. of phosphoric acid per pound of soil (2773 parts of phosphoric acid per million parts of air-dry soil) were weighed out and thoroughly mixed with the soil. The fertilized soils were treated with the different soil amendments as shown below:—

Sulphur—At two tons per acre.

Molasses—At two tons per acre.

Mohua flowers—At two tons per acre.

Green manure—Calculated to supply two per cent. green matter on the weight of the soil.

TABLE II

*$P_2O_5$ extracted by $CO_2$ saturated water from soils treated with different P-carriers and various soil amendments*

(Parts per million on moisture-free soil

| Extractions No. | Superphosphate | | | | | Rock phosphate | | | | | Bone meal | | | | | Unfertilised soil |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Soil- + super only | Sulphur | Molasses | Mohua flowers | Green manure | Soil + Rock P. | Sulphur | Molasses | Mohua flowers | Green manure | Soil- + Bone-meal | Sulphur | Molasses | Mohua flowers | Green manure | |
| 1 | 582·0 | 360·6 | 555·6 | 556·5 | 589·1 | 40·4 | 103·8 | 38·2 | 36·5 | 26·3 | 79·1 | 337·4 | 110·4 | 26·70 | 123·8 | traces |
| 2 | 306·4 | 200·4 | 322·5 | 325·6 | 373·4 | 52·2 | 74·6 | 56·6 | 50·2 | 53·8 | 172·5 | 227·1 | 133·2 | 46·00 | 163·4 | 9·41 |
| 3 | 224·5 | 176·3 | 198·7 | 223·9 | 210·3 | 60·9 | 150·7 | 61·4 | 50·7 | 58·3 | 321·8 | 328·7 | 296·2 | 102·10 | 280·1 | 17·67 |
| 4 | 147·9 | 127·2 | 129·3 | 131·1 | 118·4 | 65·3 | 160·2 | 62·4 | 80·9 | 75·7 | 335·2 | 382·6 | 300·9 | 267·10 | 316·4 | 18·91 |
| 5 | 69·9 | 63·0 | 74·3 | 76·4 | 66·8 | 62·9 | 129·4 | 50·6 | 87·4 | 78·6 | 323·6 | 240·6 | 246·5 | 228·60 | 310·1 | 29·59 |
| 6 | 54·2 | 66·5 | 60·5 | 66·9 | 56·8 | 57·9 | 94·7 | 53·1 | 71·8 | 85·4 | 305·5 | 228·6 | 232·1 | 215·80 | 292·6 | 31·96 |
| Total | 1385·0 | 994·0 | 1340·9 | 1380·5 | 1414·8 | 349·6 | 713·4 | 322·3 | 377·5 | 378·1 | 1537·7 | 1745·3 | 1319·3 | 886·4 | 1486·4 | 107·54 |
| Total as % of added | (49·9) | (35·85) | (48·2) | (49·3) | (50·4) | (12·6) | (25·7) | (11·6) | (13·5) | (13·6) | (55·5) | (62·8) | (47·3) | (31·6) | (52·9) | .. |
| pH | 5·8 | 4·7 | 5·8 | 5·75 | 5·75 | 6·63 | 5·0 | 6·62 | 6·60 | 6·60 | 6·5 | 5·0 | 6·4 | 6·4 | 6·3 | 6·8 |

After mixing the fertilizers and the amendments, the soils were brought to their moisture equivalent by the addition of the calculated quantities of water and this was maintained for a period of two and a half months.

At the end of this period requisite quantities of samples were removed for analyses which included the following determinations.

(i) Moisture.

(ii) Phosphate supplying power of soil using carbonated water as an extractant, and successively extracting the soil six times.

(iii) pH values by the glass electrode.

The results of analyses are presented in Table II and graphically represented in Fig. 1 (*a* to *e*).

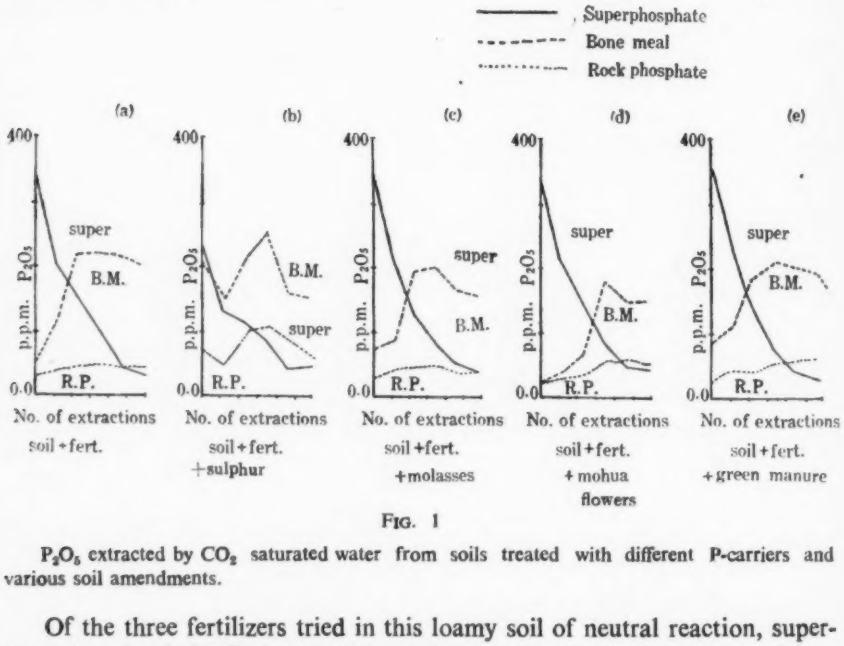



FIG. 1

$P_2O_5$ extracted by $CO_2$ saturated water from soils treated with different P-carriers and various soil amendments.

Of the three fertilizers tried in this loamy soil of neutral reaction, superphosphate is distinctly better than rock phosphate and almost as good as bone meal as judged from the amount of phosphate solubilized by six successive extractions with carbonated water. The total amount of phosphate solubilized may be expected to furnish a rough index of the availability of phosphorus under the different treatments over an extended period of time. It would take into account the effect produced by the treatments on the total supply of phosphorus that may be released for plant use during

its entire period of growth. On the other hand, the quantity of phosphate released in each successive extraction may be assumed to represent the rate of replenishment of the soil solution with phosphate ions, or in other words, the phosphate supplying power of the soil.

While the application of sulphur along with the fertilizers has increased the total available phosphate in the case of bone meal and rock phosphate to a considerable extent, it has depressed the availability in the case of superphosphate. Expressed as percentage of the total additions in the form of fertilizer there is a reduction of nearly 15 per cent. in the availability (*vide* bracketed figures in the table) of superphosphate when combined with sulphur additions and an increase of 13·1 per cent. and 7·3 per cent. in the case of rock phosphate and bone meal respectively. As regards the organic soil amendments, it may be noted that the application of molasses has depressed the availability of phosphate in all the three fertilizers, the behaviour of the other two being different with different fertilizers. Thus green manure is found to increase the availability of rock phosphate and superphosphate and decrease that in bone meal. While no change is observed in the availability of superphosphate when combined with the application of mohua flowers, considerable reduction in the availability of phosphate in bone meal is noticed, that of rock phosphate being increased to a slight extent.

It is well known that molasses contain appreciable quantities of sugars, which step up the bacterial population of soil by supplying the necessary energy giving material. It is suggested that the increased microflora as a result of its application have metabolised a part of total supply of available phosphorus thus immobilising it into complex organic forms like lecithin, phytin, etc., as suggested by Taylor (1946) and Pierre (1948).

As regards the rate of supply of phosphorus or in other words the phosphate supplying power of the soil under the three fertilizers (*vide* curve *a*), it may be noted that it shows a continuous fall in the case of superphosphate, a very slight tendency of increase in rock phosphate, and a considerable increase in the case of bone meal. The high release of phosphate ions under superphosphate in the beginning of the extractions is evidently due to the presence of large quantities of water-soluble phosphate accompanying the fertilizer which incidentally appear in the carbonated water extracts. Further, it may be of interest to note that the curves for the release of phosphate from superphosphate remain more or less the same when the fertilizer application is combined with that of all the amendments.

The application of the organic soil amendments has not materially affected the phosphate supplying power of the soil fertilized with any of the three fertilizers as indicated by the shape of curves (*c*, *d*, *e*), the fourth extraction dissolving out the maximum amount of phosphate in the case of bone meal and rock phosphate.

The behaviour of sulphur is quite different from that of the other amendments. When the application of sulphur is combined with that of superphosphate, the phosphate supplying power is appreciably depressed. This reduction in the rate of phosphate supplying power may be due to the lowering of pH values of the soil from 6·8 to 4·7 as a result of the presence of free acid accompanying the fertilizer and the production of sulphuric acid from the decomposition of sulphur. As suggested by Bradfield *et al.*, (1935) the inactivation of phosphate has been brought about by the formation of iron and aluminium phosphates at the low pH values. The shape of curves for the phosphate supplying power under bone meal and rock phosphate (Fig. *b*) is more or less the same although the curve for bone meal continues to be much higher than that for rock phosphate throughout the six extractions. In the case of both these fertilizers, the phosphate supplying power is considerably enhanced by the application of sulphur—a physiologically acid soil amendment.

## SUMMARY

In order to assess the efficiency of different P-carriers it is necessary to know not only how it affects the total supply of available phosphate but also the rate at which the phosphate is released in an available form for the use of the plants.

On the basis of the above two factors the results of these studies may now be summarised as under:—

From the point of view of the total supply of available phosphorus, superphosphate as well as bone meal are almost four times as efficient as rock phosphate in the Karjat soil. Whereas the rate of supply of phosphates goes on decreasing in the case of superphosphate, that in the case of bone meal shows a continuous increase. As regards the effect of different soil amendments when used in conjunction with the phosphatic fertilisers, the total available phosphorus as well as its rate of supply is adversely affected when sulphur is applied along with superphosphate. The organic soil amendments have not produced any effect on the total as well as on the rate of supply of available phosphorus when they are added along with superphosphate. Whereas the addition of sulphur along with either rock phos-

phate or bone meal has increased the availability of phosphorus, the organic amendments do not seem to have any effect, mohua flowers on the other hand having depressed the rate of release of phosphorus when applied with bone meal.

## References

1. Bradfield, R., Scarseth, G. and Steele, J. G. — *Trans. Inter. Cong. Soil. Sci.* (*Oxford*), 1935, **1**, 74.
2. Taylor, C. B. .. *Nature*, 1946, **158**, 447.
3. Pierre, W. H. .. *Jr. Am. Soc. Agron.*, 1948, **40**, 1.

1430-53 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

# THE EFFECT OF IONS ON THE CONTRACTILE MECHANISM OF UNSTRIATED MUSCLE AND ITS RELATION TO TONUS

By Sunita Inderjit Singh, M.D.
AND
Inderjit Singh, F.A.Sc.
(*From the Department of Physiology, Medical College, Agra*)

Received May 20, 1954

There are wide differences between the responses of various types of unstriated muscle. These differences are due to variations in their (*a*) excitatory mechanisms, (*b*) contractile mechanisms. The excitatory mechanisms differ mainly in two respects: (*a*) in their response to electrical and chemical stimulation, (*b*) in their response to excitation or inhibition. The other difference between various types of unstriated muscle are due to variations in their contractile mechanism. It is therefore important to study the contractile mechanism and obtain results which are independent of any theory of muscular contraction or relaxation.

In the present research an attempt has been made to study the effects of various substances on the contractile mechanism of unstriated muscle. The effect of various substances on the contractile mechanism of unstriated muscle has been described previously (Singh, 1943, 1944; Singh and Singh, 1949 *a*, 1950 *a*, 1950 *b*, 1951 *a*, 1953). In this method the muscle is allowed to die in the experimental solution, which is not oxygenated. It is well known that the normal permeability of the muscle depends upon metabolism, so that as the muscle dies, it becomes inexcitable as well as fully permeable to the substance; the excitatory system then ceases to function, and the contractile system acts without interference.

In the above experiments, doubt may be expressed as to whether the excitatory system had produced interference or not. In the present research, therefore, a different procedure has been adopted; the muscles were killed by heat prior to experimentation. The results with these heat killed muscles have been found to be similar to those obtained with dying muscles, though there were minor differences. The validity of the dying muscle method is therefore established; this method is superior to others as the contractile system is acted upon by various substances in as physiological state as

possible. Some experiments were also performed on glycerinised muscles; the results obtained were more or less the same as with the other two methods, though the contractile system in glycerinised muscles appeared to be less responsive than in dying or heat killed muscles.

## EXPERIMENTAL

The experimental procedure has been described previously (Singh and Singh, 1953); it has been slightly modified in the present research. Dogs were anæsthetised with chloroform and frogs, *Rana tigrina*, were killed with a blow on the head; their stomachs were removed and opened at the lesser curvature. Transverse pieces were taken and the mucosa removed. The temperature of the saline was gradually raised from room temperature to 50° C. in about 5 minutes and kept for 5 minutes at that temperature if dog's stomach muscle was used, and for one minute if frog's stomach muscle was used. This was done because dog's stomach muscle becomes irresponsive if kept at 50° C. for 4 minutes and frog's muscle, if kept for less than a minute; these differences between the two kinds of muscle are probably due to the fact that the pieces of dog's muscle are thicker than those of frog's muscle and hence take longer time to come into equilibrium with the saline.

Two sets of experiments were performed, one for testing active and the other for testing passive relaxation. For active relaxation, unloaded frog's stomach muscle was used, as it is most suitable. For passive relaxation, dog's stomach muscle was used; the muscles were suspended in saline under a constant load of 7–8 g. When the muscles were loaded, they inevitably lengthened and with the small load used, they reached a constant length in an hour's time. Their length was then measured between the ligatures; after immersion in the experimental solution for 24 hours, the length was again measured and expressed as per cent. of the initial length.

As in the previous paper (Singh and Singh, 1953), the length of loaded muscles in distilled water was taken as the standard. Muscles with shorter length (expressed as p.c. of the length) were considered to be relatively contracted and those with greater length relatively relaxed, as no matter whether the muscle contracts or relaxes, it lengthens when loaded.

A few words about the effect of load on dog's stomach muscle are necessary here. The final length of a loaded muscle is determined by two factors: (1) the load, (2) state of contraction. The greater the load, the greater the final length. If the muscle is contracted, the final length under the same load decreases; if the muscle relaxes, the opposite happens. The final length, therefore, is determined by two opposing forces, the contractile

tension of muscle and the load. In the experimental procedure, outlined above, it is therefore justifiable to presume that a muscle which lengthens less, is contracted, relatively to the other which lengthens more, provided a constant load has been used. The load can only be considered constant if the cross-section of the various pieces of muscles is the same. This is very difficult to realise in practice, but it was found that pieces from the same stomach, if not taken from very near the cardiac or pyloric ends, gave remarkably consistent results, if care was taken to cut them approximately of the same cross-section, and a light load was used, as in our experiments.

Dying and glycerinised muscles were also used for comparison in some experiments.

## Results

### *Active Relaxation*

*Effect of temperature.*—Heat is a common agent that denatures proteins. Its effect on unstriated muscle is most interesting. When circular strips of frog's stomach muscle are heated to 50° C., they contract by 2 to 5 per cent.; this slight contraction might be due to collagen, which however will dissolve at higher temperatures. Further heating up to 60° C. causes them to actively lengthen by 30 to 80 per cent. before they are coagulated; cogulation causes the muscle to contract. These experiments suggest that the process of active relaxation is similar to that of denaturation of proteins. This view is further supported by the fact that the substances which cause denaturation of proteins, also cause similar relaxation (Singh and Singh, 1954). Here, by active relaxation of dead muscle is implied relaxation of unloaded muscle.

Some muscles behave differently. They lengthen by 10 to 30 per cent. at 50° C. and lengthen further with heating up to 60° C. This happens with frog's stomach muscle, guinea pig's uterus, longitudinal muscle of dog's stomach and intestine, rabbit's small intestine. Circular muscle strips from the pyloric end of dog's stomach contract by about 30 per cent. at 50° C. if unloaded, and regain their original length only partially by further heating. If dog's stomach muscle is loaded, then it relaxes if heated beyond 50° C. This shows that there is some difference between the contractile mechanisms of frog's and dog's stomach muscle. Glycerinised muscles do not relax actively if heated.

These experiments show that relaxation is of two kinds, one active and the other passive. Both are akin to denaturation of proteins, but in active relaxation there is some further process which causes repulsion between adjacent parts of the unfolded peptide chains.

*Effect of urea and allied substances.*—Urea and urethane cause active relaxation of dying unstriated muscle (Singh and Singh, 1949 *a*, 1950 *b*). The question arises whether urea acts through the excitatory mechanism or directly on the contractile mechanism. Active relaxation of unstriated muscle through the excitatory mechanism such as produced by adrenaline, is abolished by cyanide (Singh and Singh, 1951 *b*); sodium cyanide (1 in 5000) has no effect on active relaxation produced by urea. Similarly heat killed frog's stomach muscle actively relaxes in urea solutions, the concentrations used being 0·25, 0·50, 0·75, 1, 2, 3, 4, 5, 6 and 7 *M* (12 experiments). Higher concentrations such as 30, 40, 60 per cent. also produce active relaxation; in 60 per cent. urea, the muscles begin to disintegrate if handled.

The above experiments also support the view that the process of active relaxation is similar to denaturation of proteins. There is one difference, however, between the two phenomena. Denaturation of proteins is produced by high concentrations of urea—40 to 60 per cent.; active relaxation of unstriated muscle can be produced by much smaller concentrations. High concentrations of urea would undoubtedly cause denaturation of the muscle proteins; similar action of small concentrations may be due to the probability that in muscle the molecules or part of the molecules of the contractile proteins are already aligned to unfold.

It is possible that the action of small concentrations of urea is different from that of high concentrations; it may have something to do with polymerisation of actin. Small concentrations of urea, 5 to 15 per cent., cause reversible polymerisation of actin, the action of high concentrations, 30 per cent., being irreversible (Szent-Gyorgyi and Joseph, 1951). That urea acts by polymerising actin appears unlikely as other substances, such as 0·1 *M* potassium iodide, which cause polymerisation of actin, do not cause active relaxation.

The effect of urea and similar substances would point towards those bonds in and between protein molecules which are tentatively considered as hydrogen bonds; the contractile proteins therefore dissociate at these bonds during active relaxation. According to Haurowitz (1950), the denaturing action of concentrated solutions of urea is probably due to the formation of hydrogen bonds with the peptide chains. The small polar urea molecules are probably driven by their thermal movements as wedges between the peptide chains, cleaving the hydrogen bonds and being bound to the liberated peptide linkages by the formation of new hydrogen bonds. In this way the peptide chains are unfolded and the proteins denatured.

According to the modern theory, the contraction and relaxation of muscle are due to folding and unfolding of the contractile proteins. Thus denaturation of proteins and muscular relaxation appear to have a common basis on the molecular level.

The above view is further supported by the fact that glycerinised muscles do not relax actively in urea solutions. Glycerine is known to protect proteins from denaturation. The rate of denaturation is probably reduced by the loose association of glycerol molecules with the protein, due to polar hydroxyl groups of glycerol (Haurowitz, 1950). Denaturation of proteins is also prevented by solutions containing 80–90 per cent. acetone; acetone also prevents active relaxation of frog's unstriated muscle by urea. Denaturation of proteins is inhibited by glucose and other sugars (Brosteaux and Erickson-Quensel, 1935); glucose and sucrose (0·224 *M*) also inhibit active relaxation of frog's unstriated muscle by urea.

Formamide and acetamide (0·224 *M*) produce active relaxation of frog's stomach muscle, their action being similar to that produced by urea. These substances are also known to cause denaturation of proteins. They do not cause active relaxation of glycerinised muscle.

Small concentrations (0·224 *M*) of urea, formamide and acetamide cause active relaxation of living muscle, the action being reversible. Thus frog's stomach muscle may actively relax up to 200 per cent. of its initial length in one hour's time. The muscle after reimmersion in saline is found to respond to stimulation. This shows that active relaxation of dead muscle in urea solutions represents the normal mode of such relaxation in living muscle. High concentrations, such as 30 to 60 per cent. cause active relaxation which is irreversible.

*Effect of distilled water.*—Distilled water causes active relaxation of dying and living muscles (Singh, 1944; and Singh and Singh, 1949 *a*). It causes active relaxation of heat killed frog's muscle, so that the action is on the contractile mechanism. This is further shown by the fact that 1 in 10,000 sodium cyanide does not prevent the active relaxation. Glycerinised muscles do not relax actively in distilled water.

*Effect of sodium cyanide.*—Sodium cyanide, 0·112 *M*, causes active relaxation of dying muscle (Singh and Singh, 1949 *a*); it has similar action on heat killed or glycerinised muscles. It acts, therefore, on the contractile mechanism. The action of cyanide on frog's stomach muscle is therefore in marked contrast to that of *Mytilus* muscle, the contractile mechanism of which is caused to contract (Singh and Singh, 1951 *a*).

The actions of sodium cyanide and barium chloride clearly show that there are two kinds of contractile mechanisms in unstriated muscle. Both these mechanisms are responsible for tonus which does not require energy. In *Mytilus* muscle, this tonus appears to be due to a process opposite to that of denaturation; the fibrous contractile protein appears to undergo a globular transformation or renaturation (Singh and Singh, 1951 *a*).

*Effect of acids and alkalies.*—Acids and alkalies cause active relaxation of dying muscle (Singh and Singh, 1949 *a*), they have similar action on heat killed or glycerinised muscles; according to Haurowitz (1950), the denaturing action of mineral acids and alkali hydroxides is due to their action on the ionic groups of the proteins. Mineral acids convert the negative $—COO^-$ groups into —COOH groups, so that the salt bridges $—COO^- \cdots H_3N$ are cleaved and the peptide chains unfold; the positive charges are similarly abolished by alkali.

*Effect of adrenaline and acetylcholine.*—Adrenaline and acetylcholine have no action on heat killed unstriated muscle.

*Passive Relaxation*

Loaded heat killed dog's stomach muscle lengthens more than the dying muscle in various solutions. Thus the length of 6 dying muscles immersed in Weber-Edsall solution for 24 hours was 105 ± 5 per cent. of the initial length, and that of heat killed muscle was 133 ± 12 per cent. Heating up to 50° C. therefore loosens somewhat the internal structure of the muscle proteins as denaturation takes place.

Heat killed muscle behaves somewhat differently than dying muscle. Dying muscle may contract in various solutions. Heat killed muscle contracts very little, by 2 per cent., if at all in 0·2 *M* KCl, so that the effect of various solutions on the heat killed muscle is on the extent of relaxation. If the dying muscle is previously poisoned with 1 in 10,000 sodium cyanide, then contraction does not occur, and so the effect of various substances that has been described, is on relaxation only. The contraction that actually occurs in the dying muscle is therefore, through the excitatory system.

*Effect of potassium chloride.*—The effect of potassium chloride solutions containing 0·001 *M* magnesium chloride, on heat killed muscle resembles that on dying muscle (Singh and Singh, 1953). Potassium chloride produces two or three kinds of relative contraction. Contraction occurs in 0·1 *M* KCl, or in 0·1 to 0·3 *M* KCl (Fig. 1). It begins in 0·04–0·06 *M* KCl and relaxation occurs between 0·1 and 0·2 *M* KCl or between 0·2 and 0·3 *M* KCl

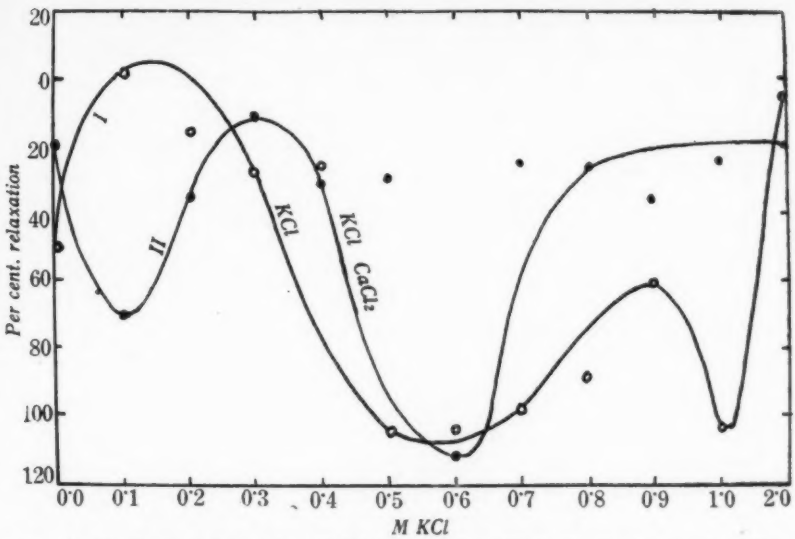



FIG. 1. Dog's stomach muscle. Killed by heating at 50° C. for 5 minutes. I. Effect of potassium chloride. II. Effect of calcium chloride, 0·01 *M* $CaCl_2$, on the effect of potassium chloride. Muscle immersed for 24 hours.

(about 0·26 *M* KCl). A further contraction may occur between 0·6 and 1 *M* KCl. Very high concentrations, 2 *M* KCl, cause contraction.

Calcium chloride, 0·01 *M* $CaCl_2$, has differential effect on these contractions (Fig. 1). It antagonises the contraction in 0·1 *M* KCl, but promotes or has no effect on the other two. It has been suggested that the contraction in 0·1 *M* KCl corresponds to superprecipitation of actomyosin (Singh and Singh, 1953). The action of calcium supports this suggestion, as calcium inhibits superprecipitation. The action of calcium is antagonised with further increase in the concentration of magnesium, as occurs with superprecipitation of actomyosin. The action of potassium chloride was also tested on glycerinised muscle; 0·1 *M* KCl causes the maximum contraction as in dying or heat killed muscle (Fig. 2).

The action of sodium chloride resembles that of potassium chloride (Fig. 3). The effect of other salts such as LiCl, $NH_4Cl$, KBr, $KNO_3$ KI, KSCN, KCN, $CaCl_2$, $SrCl_2$, $BaCl_2$, $MgCl_2$ was tested. The effects were irregular and difficult to interpret.

## DISCUSSION

*Intimate nature of muscular contraction.*—According to modern views, denaturation consists of a rearrangement of the peptide chains in the

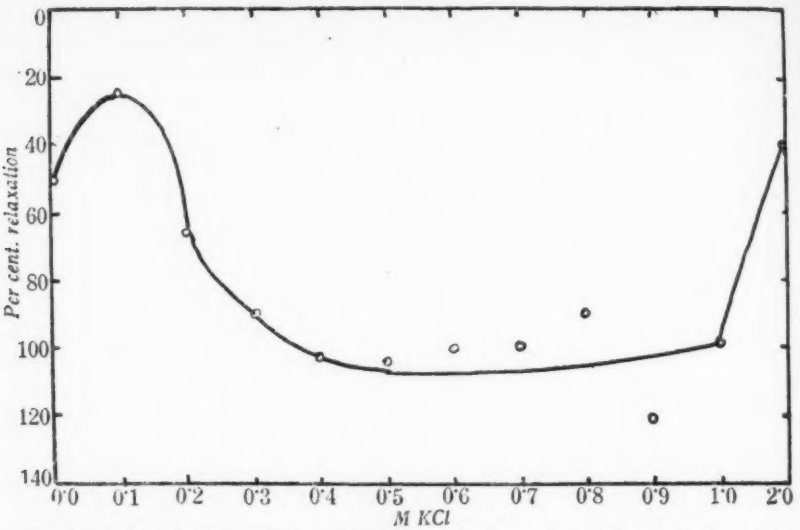



FIG. 2. Dog's stomach muscle. Glycerinised. Effect of potassium chloride. Muscle immersed for 24 hours.

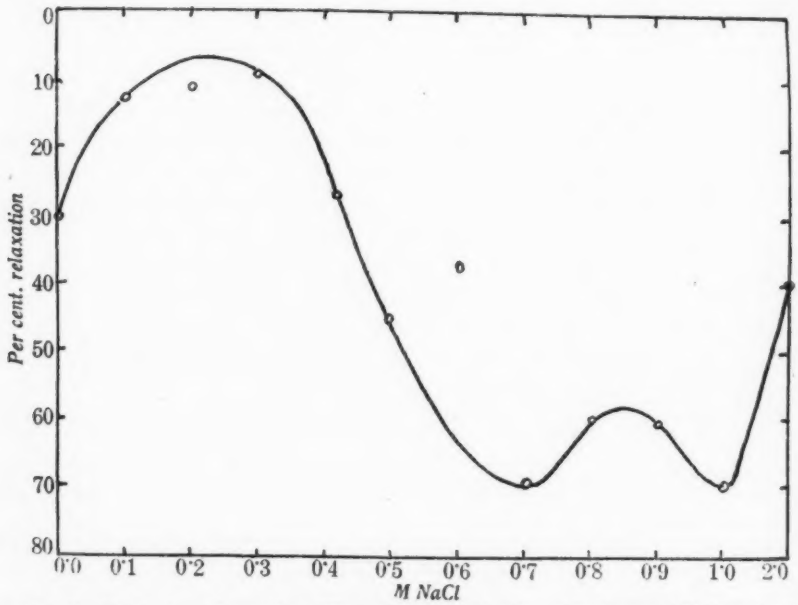



FIG. 3. Dog's stomach muscle. Effect of sodium chloride. Muscle was killed by heating at 50° C. for 5 minutes.

protein molecule due to rupture by the denaturing agent of the weak bonds. It is presumed that denaturation consists of an alteration of the specific internal structure of the protein, wherein the closely folded peptide chains unfold (Haurowitz, 1950). The modern view of contraction of muscle is also similar; it is presumed that contraction is due to folding of the contractile protein. The process of relaxation of muscle would therefore correspond to that of denaturation. The active relaxation of unstriated muscle by heat and other denaturing agents may be considered as a "visible" demonstration of the unfolding of peptide chains during denaturation. These experiments therefore elucidate the nature of both denaturation of proteins and the intimate nature of muscular contraction.

*Contractions of unstriated muscle.*—The effect of potassium chloride shows that only one kind of contraction of unstriated muscle corresponds to superprecipitation of actomyosin. Csapó (1948, 1950) has made estimations of myosin and actomyosin content of uterus muscle. They are much less than in striated muscle. The actomyosin content rises during pregnancy, but even in labour, uterus muscle has a much lower actomyosin content than striated muscle. Kováts (1949) found more myosin in the ventricles than in the auricles. The result of these estimations show that the actomyosin content of these muscles varies with contractility. But if the actomyosin content of various muscles should vary with their contractility, then unstriated muscle should contain the most actomyosin, as it is constantly contracted; actually it contains the least. Therefore, one kind of contraction of unstriated muscle does not depend upon actomyosin.

The above conclusion is also supported by energy considerations. Unstriated muscle contains about one-seventh of the high energy phosphates in striated muscle, but the energy requirements for a single phasic contraction appear to be of the same order of magnitude in both the muscles (Csapó and Gergely, 1950); it follows therefore, that a greater part of the sustained contraction of unstriated muscle could not be due to ATP, and hence could not correspond with superprecipitation of actomyosin. It appears therefore, that only the phasic contraction of unstriated muscle is produced by ATP and superprecipitation of actomyosin; tonus which requires energy is also probably due to the same chemical changes.

*Tonus in unstriated muscle.*—The effect of sodium cyanide and barium chloride clearly shows the existence of two kinds of contractile mechanisms in unstriated muscle; one of these is present in *Mytilus* muscle and the other kind in mammalian and frog's unstriated muscles, so far investigated. Therefore there are two distinct mechanisms of tonus in unstriated muscle.

If relaxation of muscle corresponds to denaturation of proteins, then contraction would be an opposite process, that is renaturation or globular transformation in the protein molecule. There is direct evidence for such transformation in *Mytilus* muscle (Singh and Singh, 1951 *a*). In solutions of barium chloride and sodium cyanide there is contraction of dying *Mytilus* muscle. The muscle goes on swelling in a globular fashion, but is unable to relax, in marked contrast to frog's stomach muscle which lengthens out enormously; in the former therefore, there must be globular transformation in the contractile protein.

During contraction, increase in folding requires energy, but if the muscle is allowed to shorten, it may be presumed that new bonds develop, as in the globular protein, which keep the folded peptide chains together. This is shown in Fig. 4: (i) shows the relaxed state of muscle; (ii) shows contraction which requires energy; (iii) shows development of cross linkages,

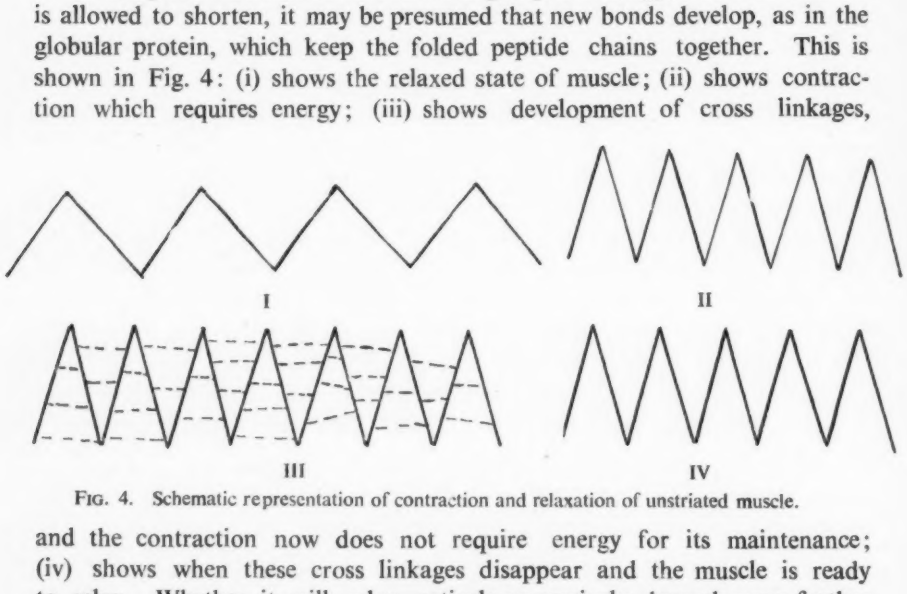



FIG. 4. Schematic representation of contraction and relaxation of unstriated muscle.

and the contraction now does not require energy for its maintenance; (iv) shows when these cross linkages disappear and the muscle is ready to relax. Whether it will relax actively or passively, depends upon further developments.

The above view is supported by several observations on unstriated muscle. The muscle may be unable to lift a weight, but if it is allowed to shorten by lifting the lever with the hand, it will now keep the weight suspended at the shortened length; this suggests that the folding of the polypeptide chains permitted strategic points in the chain to come near enough for the formation of cross linkages. The opposite of this also holds true. If the muscle is forcibly stretched, then it may not recover its original length on release. The muscle may thus be permanently lengthened, suggesting that the cross linkages had been damaged. The interesting point is that

the phasic contraction may not be affected. This shows that these cross linkages are only formed for the tonic contraction, though there may be different ones for the phasic contraction. The presence of stronger intermolecular and intramolecular linkages in unstriated than in striated muscle, is suggested by the fact that the dynamic modulus is greater in the former than in the latter (Pryor, 1950). Further coagulated muscle contracts strongly and exerts considerable tension. Coagulation is supposed to be due to the formation of intermolecular salt bridges between ionic groups which are displaced to the surface of the particle through the unfolding of the polypeptide chains (Haurowitz, 1950).

Oxygen consumption measurements of unstriated muscle also support the above scheme. Oxygen usage is markedly dependent upon the length of the muscle. During isotonic contraction, if the muscle is allowed to shorten, then the oxygen consumption decreases (Lovatt Evans, 1923), but it was first shown by Rao and Singh (1940) and recently by Bulbring (1953), that if the muscle is not allowed to shorten, as under isometric conditions, then the oxygen consumption increases both during tonic and phasic contractions. All these facts can be explained in the light of findings in this paper, that the process of relaxation of muscle is similar to that of denaturation of proteins, and that globular transformation occurs during contraction. If the muscle is allowed to shorten, then the development of cross linkages will be facilitated, and hence the contraction will not require any energy for its maintenance.

The cross linkages are of two kinds; one kind is found in *Mytilus* muscle and the other in mammalian and frog's unstriated muscle. Both these linkages are dissolved by potassium chloride and strengthened by sodium chloride and hydrogen ions. As the sodium content of unstriated muscle is greater than that of striated muscle, the instrinsic tone and viscosity of unstriated muscle is due to its sodium content; the formation of these cross linkages will therefore be facilitated by the entrance of sodium and production of carbon dioxide and lactic acid during activity. The cross linkages are dissolved by sodium cyanide in mammalian and frog's muscle, but not in *Mytilus* muscle. The varying action of cyanide on various types of muscle suggests the occurrance of intermediate types. The occurrence of phasic contraction would suggest the dissolution of these " tonic " linkages prior to the contraction, thus accounting for the latency relaxation. As such tonus is very little marked in striated muscle when compared to unstriated muscle, the latency relaxation is more marked in the latter.

Tonus in unstriated muscle is of two kinds. One kind requires energy and its mechanism must be more or less the same as that of the phasic

contraction. The mechanism of tonus which does not require energy, differs in various types of unstriated muscle owing to two factors: (1) The difference in the nature of cross linkages. (2) The difference in the nature of relaxation, which may be active or passive. Adrenaline causes active relaxation of frog's stomach muscle and guinea pig's uterus, but not that of rabbit's gut muscle, which is otherwise very sensitive if allowed to relax passively. In *Mytilus* muscle, we have not noticed active relaxation. The formation of cross linkages for the maintenance of tonus without expenditure of energy, would be necessary in that mechanism in which relaxation is passive. In these muscles, therefore, the contractile system for phasic contraction has been modified for the development of tone. The function of active relaxation is either to reinforce passive relaxation or it represents a separate contractile mechanism; the repulsive groupings may be in the same polypeptide chain that relaxes passively or in a different one.

The above facts bring all the present theories of tonus in unstriated muscle under one fold. There are three such theories: (1) The tetanus or the viscosity theory. (2) The active relaxation theory, tonus being due to retarded relaxation. (3) The catch mechanism theory. It appears that all these theories are correct; unstriated muscle is a very complicated structure. The formation of intermolecular and intramolecular linkages will lead to increase in viscosity, thus accounting for the viscous tone and slow tetanus. The folding of polypeptide chains by electric forces will facilitate movement in one direction and retard in the other, thus accounting for the catch mechanism. Active relaxation has been shown to occur. Contraction is similar to coagulation, as required by Heilbrunn's theory.

## SUMMARY

1. A new technique is described to determine the action of substances on the contractile mechanism of unstriated muscle. It consists in killing the muscle by heating it to 50° C. for a few minutes.

2. The results obtained on heat killed muscle are more or less similar to those on dying muscle with minor differences.

3. Active relaxation occurs if the muscle is heated to 50–60° C. As heat denatures the proteins, it appears that the process of relaxation of muscle is similar to that of denaturation of proteins. Most substances that denature proteins, cause unstriated muscle to relax actively. These experiments therefore throw light both on the process of relaxation and on that of denaturation of proteins.

4. Urea, sodium cyanide, distilled water, formamide, acetamide, acids and alkalies cause active relaxation of heat killed muscle.

5. Potassium chloride has similar action on heat killed muscle as on dying muscle. It causes two or three kinds of contraction. One of these is antagonised by calcium, and probably corresponds to superprecipitation of actomyosin. The other contractions are probably due to some other proteins.

6. The effect of sodium chloride resembles that of potassium chloride.

7. Possible mechanisms of tonus are discussed.

## References


Brosteaux, T. and B. Erickson-Quensell — *Arch. Phys. biol.*, 1953, **12,** 209 (Quoted from F. Haurowitz, *The Chemistry and Biology of Proteins*, 1950, Academic Press, Inc., New York).

Bulbring, E. .. *J. Physiol.*, 1953, **122,** 111.

Csapò, A. .. *Nature*, 1948, **162,** 218.

———— .. *Am. J. Physiol.*, 1950, **160,** 46.

Csapò, A. and Gergely, J. .. *Nature*, 1950, **166,** 1078.

Haurowitz, F. .. *Chemistry and Biology of Proteins*, 1950, Academic Press, New York.

Kovàts, J. .. *Nature*, 1949, **163,** 606.

Lovatt Evans, C. .. *J. Physiol.*, 1923, **58,** 22.

Pryor, M. G. M. .. *Progress in Biophysics*, 1950, Butterworth-Springer Ltd., London.

Rao, M. S. and Singh, I. .. *Ibid.*, 1940, **98,** 12.

Singh, I. .. *Ibid.*, 1938, **94,** 322.

———— .. *Proc. Ind. Acad. Sci.*, 1943, **18,** 53.

———— .. *Curr. Sci.*, 1944, **13,** 311.

Singh, S. I. and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1949*a*, **30,** 270.

———— .. *Ibid.*, 1949*b*, **30,** 343.

———— .. *Ibid.*, 1950*a*, **31,** 351.

———— .. *Ibid.*, 1950*b*, **32,** 12.

———— .. *Ibid.*, 1951*a*, **33,** 165.

———— .. *Ibid.*, 1951*b*, **33,** 184.

———— .. *Nature*, 1951*c*, **168,** 829.

———— .. *Proc. Ind. Acad. Sci.*, 1953, **37,** 188.

———— .. *Curr. Sci.*, 1954, **23,** 126.

Szent Gyorgyi, A. G. and Joseph, R. — *Arch. Biochem. Biophys.*, 1951, **31,** 97 (Quoted from D. M. Needham, *Advances in Enzymology*), 1952, Interscience Publishers, New York.

# THE NEUROSECRETORY SYSTEM OF THE FRUIT FLY *CHÆTODACUS CUCURBITÆ* Coq.

## I. Distribution and Description of the Neurosecretory Cells in the Adult Fly

BY DR. K. K. NAYAR

(*Department of Zoology, University College, Trivandrum*)

Received October 4, 1954

(Communicated by Dr. C. S. Venkateswaran, F.A.SC.)

### INTRODUCTION

NEUROSECRETORY cells, *i.e.*, nerve cells showing well-developed glandular activity, have been discovered in many groups of insects (*vide* Scharrer, 1954). These cells are situated in different parts of the central nervous system. Generally the insectan neurosecretory system has two parts: (i) Special cells of the protocerebrum and (ii) the axonic system forming the nervi corporis cardiaci and allati and the corpora cardiaca and allata. The corpora cardiaca and the corpora allata, originally described as the ganglia of the insectan sympathetic system, have recently been shown to be endocrine glands by various workers (review, Scharrer, 1948, 1953). In addition to the parts mentioned above, neurosecretory cells have been discovered in the frontal ganglion, sub-œsophageal ganglion, metathoracic and abdominal ganglia (Day, 1940; Scharrer, 1941; Nayar, 1953). The functional significance of the cerebral neurosecretory cells have been discussed by Scharrer (1952) in *Leucophæa maderæ*; by Thomsen (1952) in *Calliphora erythrocephala* and by Williams (1952) in *Platysamia cecropia.*

In this paper, an account is given of the neurosecretory cells seen in the brain, thoracic and abdominal ganglia of the fruit fly *Chætodacus cucurbitæ* Coq. (Trypetidæ: Diptera). A general description of the neurosecretory cells appeared to be desirable because of their specially characteristic distribution in the thoracic ganglion, forming a more conspicuous cluster than in the brain.

### MATERIAL AND METHODS

Late pupæ (pupæ from which adults were about to emerge) and adult flies aged 1 to 4 days after emergence from puparia were used for study. The larvæ were reared on fruits of *Trichosanthus anguina* (snake gourd).

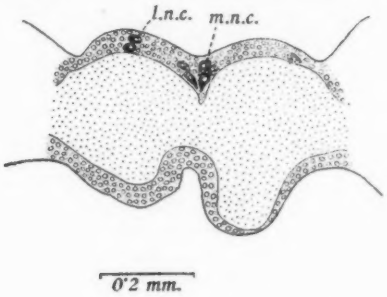
For examination of live cells, ganglia were dissected out in insect Ringer and were mounted on slides in the same solution. The cells continued

to live for more than an hour and were examined under the phase contrast and dark ground illumination.

The adults and pupæ (after removal from puparia) were fixed in Carl's, Carnoy's, Bouin's and Helly's fixatives. Material was double embedded by Peterfi's method using 1½ to 2% celloidin in methyl benzoate and sectioned at 5 and 8 to 10 $\mu$. Heidenhain's iron hæmatoxylin, Groat's hæmatoxylin, Gomori's chrome-hæmatoxylin-phloxin and Heidenhain's Azan were the stains used. Gomori's method and Azan after Bouin fixation gave the best results.

## Observations

Situated in the pars intercerebralis (Text-Fig. 1) is a cluster of about a dozen cells which show well-defined secretory activity. These form the



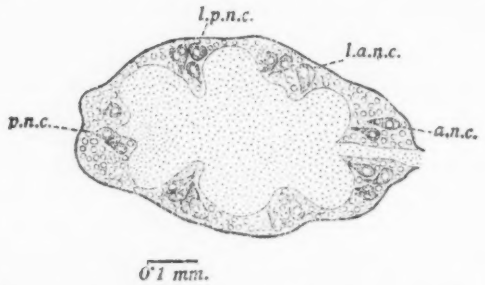

Text-Fig. 1. Camera lucida drawing (composite from a few adjacent serial sections) of a transverse section of the brain of adult *Chætodacus cucurbitæ* showing the median and lateral neurosecretory cells. *mnc.*, median neurosecretory cells; *lnc.*, lateral neurosecretory cells.

median cerebral neurosecretory cells. These cells have conspicuous axons traceable for a fairly long distance (Plate III, Fig. 1) and filled with secretory products. A few scattered cells in the lateral region of the brain form the lateral cerebral neurosecretory cells. These are situated near the region where the cerebral ganglia laterally carry the optic lobes. Both the sets consist of conspicuously large cells having large, more or less rounded nuclei with loosely arranged chromonemata and composite nucleoli stainable red in both Azan and Gomori's stains. The cytoplasm is coloured blue in chrome-hæmatoxylin and shows a reddish tinge in Azan due to the presence of numerous granules.

Large cells are distributed on the ventral side of the nerve ring also, but these do not show the blue granules in the cytoplasm. Here the granules are seen to be phloxinophil.

The nerve springing from the brain forming the nerves corporis cardiaci also is coloured blue due to the blue granules which could be traced into the corpus cardiacum below.

The neurosecretory cells of the thoracic ganglion are the most conspicuous ones (Plate III, Fig. 2). They show a definite arrangement in this composite ganglion. The single ganglionic mass is really composed of three ganglia fused together as is evidenced by the trilobed appearance of the neuropile in the centre. The neurosecretory cells are distributed along the margin of the ganglionic mass. There are four clusters (Text-Fig. 2):



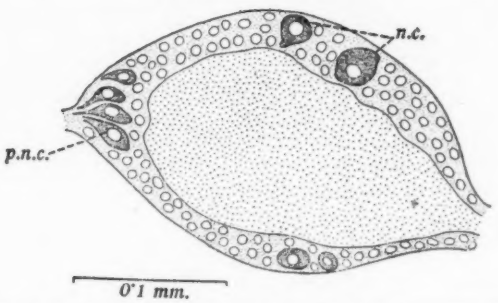

TEXT-FIG. 2. Camera lucida drawing (composite from a number of sections) of the horizontol section of the thoracic ganglion of the adult *Chætodacus cucurbitæ*, to show the distribution of the neurosecretory cells along the margin. *anc.*, anterior neurosecretory cells; *lanc.*, lateral anterior neurosecretory cells; *lpnc.*, lateral posterior neurosecretory cells; *pnc.*, posterior neurosecretory cells.

(1) an *anterior pair* of comparatively narrow, elongated cells arranged in two groups on the two sides of the connective from the sub-œsophageal mass; (2) a *posterior cluster* of cells forming a more or less continuous mass not quite separated into pairs, situated near the posterior end of the ganglion; (3) and (4) paired groups of comparatively larger cells placed laterally and in between the fibrous neuropiles (forming *lateral anterior* and *lateral posterior pairs*).

The nuclei of the thoracic neurosecretory cells are large, with characteristic chromonemata, chromocenters and nucleoli, which latter stain brilliant red in Azan. The cytoplasm is filled with granules which colour dark bluish-grey in chrome hæmatoxylin; in Azan they are reddish-dark grey. The granules which are blue or light blue extend along the axons also.

The neurosecretory cells of the single, small, abdominal ganglion are of special interest, because the few cells that are seen here show dark blue cytoplasmic products which have the appearance of colloids and fine

droplets and not quite that of fine granules (Text-Fig. 3). These cytoplasmic colloids show deeper blue colouration in chrome-hæmatoxylin-phloxin. A few are laterally placed and the other cells are lodged in the




TEXT-FIG. 3. Camera lucida drawing of a horizontal section (composite from a few adjacent sections) of the abdominal ganglion of adult *Chætodacus cucurbitæ*. *nc.*, neurosecretory cells; *pnc.*, postesior neurosecretory cells.

posterior part of the ganglion. The latter have the secretory products running into the nerve going posteriorly and this nerve in the sections show the blue granules among the fibres. These regions are probably the cut portions of the axons emerging from the neurosecretory cells. A main nerve supplies the mid and hind gut of the fly.

## EXAMINATION OF LIVE CELLS

The thoracic ganglion when removed into Ringer's solution, is a composite single structure, and when gently pressed it becomes somewhat trilobed showing its three-segmented nature. It is not elongated, but more or less rounded and somewhat flattened. Associated with the ganglion are two conspicuous sac-like tracheæ which supply its lateral edges with numerous fine tracheoles. This tracheal supply indicates that the ganglion is particularly active in these regions. Such tracheal supply is usually seen in association with neurosecretory tracts.

The neurosecretory cells are large, more or less laterally distributed forming four clusters. They measure about 36 $\mu$ in length and about 18 $\mu$ in diameter in the swollen basal region. The larger cells may measure up to 60 $\mu$ in length. The comparatively smaller cells anteriorly and posteriorly measure on an average 28 $\mu$ in length. In course of time (after about 30 minutes) when cells are still alive, due to pressure and flowing movements of the cytoplasm, the cells become flattened or enlarged. Then the large cells may sometimes reach up to a length of about 90 $\mu$.

Under phase contrast, the cell shows densely granular cytoplasm, large rounded nucleus with a lower refractive index lying towards the large swollen (abaxonal) part, with dark, usually eccentric nucleolus. The cytoplasm shows dark granules and small vacuoles of varying sizes. These small vacuoles are sometimes clustered together. In the large cells, the vacuolar system of the secretory products are seen in the abaxonal part of the cell. Here they sometimes become contiguous and finally fuse and break off as a globule of secretory material. These vacuoles and droplets are more or less transparent and dark-rimmed. Smaller spheroids of a distinctly different type are scattered in the cytoplasm. The dark granules which probably represent the mitochondria are present in the droplets and vacuoles and in the cytoplasm proper of the cell.

In phase preparations, cells in connection with the nerve could be sometimes obtained. In preparations of the abdominal ganglion showing this connection, the cytoplasmic poducts from the neurosecretory cells are seen to flow into the nerve. The same picture could be obtained by examining the preparation under dark-ground illumination; here the cytoplasmic products which appear bluish-white and translucent are seen to move into the axons which run into the nerve.

## DISCUSSION

The cerebral neurosecretory cells composed of the median and lateral clusters and the close association of the median ones with the corpus cardiacum in *Chætodacus cucurbitæ* resemble the condition observed in other dipterous insects (*vide* M. Thomsen, 1951; E. Thomsen, 1952, 1954; Scharrer, 1952; Possompes, 1953, 1954). Some workers have discovered the presence of the neurosecretory cells in the other ganglionic centres of the central nervous system. Scharrer (1941) has described the neurosecretory centres of the sub-œsophageal mass of the cockroach, Day (1940) has described the neurosecretory cells of the abdominal ganglia of Lepidoptera, and Nayar (1953) has observed the presence of these in the metathoracic ganglion of the bug *Iphita limbata*. The presence of conspicuous neurosecretory cells in definite tracts in the thoracic ganglion of *Chætodacus cucurbitæ* is very interesting. The distribution of these cells recall a resemblance to the large cells described by Matsumoto (1954) in the crab *Eriocheir japonicus*. In *Chætodacus* the axons from the thoracic cells could not directly be traced to nerves, but in certain cases they traverse the nueropile and end somewhat blindly. Whether these form neurohæmal clusters, as suggested by Carlisle and Knowles (1953) in the case of Crustacea, could be stated only after an intensive study of the nerve endings by special methods.

The neurosecretory cells of the abdominal ganglion in *Chætodacus* in the posterior region supply secretory products through the nerves, probably activating the mid and hindgut.

## Summary

1. The neurosecretory system of the adult *Chætodacus cucurbitæ* (Trypetidæ: Diptera) consists of the cerebral, thoracic and abdominal centres of secretory neurons.

2. The cerebral neurosecretory centres consist of the median and lateral neurosecretory cells of the pars intercerebralis. The cells show characteristic tintorial properties when stained in chrome-hæmatoxylin-phloxin and in Azan, recalling the nature of similar cells in other insects.

3. The axons of the median cerebral neurosecretory cells are fairly long, and the nervi corporis cardiaci and corpus cardiacum show granules similar to those observed in the cytoplasm and axons of the cells.

4. The thoracic ganglion shows a more conspicuous set of neuro-secretory cells. They consist of an anterior, lateral anterior, lateral posterior and posterior cell clusters, which in the first three are paired.

5. The single abdominal ganglion contains a few neurosecretory cells also showing characteristic tintorial properties.

6. The nerve emerging from the abdominal ganglion and going to the gut shows blue granules traceable from the neurosecretory cells at the posterior end of the ganglion.

7. Phase contrast observations of the cells show that the neuro-secretory cells of the fly have a general structure with prominent cyto-plasmic products in the form of dark granules and large and small spheroids and globules.

8. Measurements of the thoracic neurosecretory cells, which forms the larger cells of the system, have been given.

## Acknowledgments

The author wishes to express his gratitude to Prof. K. Bhaskaran Nair, Head of the Department of Zoology, University College, Trivandrum, for all facilities given and for reading through the manuscript. He is indebted to his colleague Mr. R. Parameswaran for the drawings and for help in photography.

## REFERENCES


Carlisle, D. B. and Knowles, F. G. W. "Neurohæmal organs in crustaceans," *Nature*, 1953, **172**, 404.

Day, M. F. .. "Neurosecretory cells in the ganglia of Liepidoptera," *Ibid.*, 1940, **145**, 264.

Matsumoto, K. .. "Neurosecretion in the thoracic ganglion of the crab *Eriocheir japonicus*," *Biol. Bull.*, 1954, **106**, 60–8.

Nayar, K. K. .. "Neurosecretion *Iphita limbata*," *Curr. Sci.*, 1953, **22**, 149.

Possompes, B. .. "Recherches experimentales sur le determinisme de la metamorphose de *Calliphora erythrocephala*," *Arch. Zool. Exp. Gen.*, 1953, **89**, 203–364.

———— .. "Donnees experimentales sur l'activation de l'anneau de Weismann par le cerveau chez *Calliphora erythrocephala*," *Publ. Staz. Zool. Napoli*, 1954, Suppl. **24**, 59–62.

Scharrer, B. .. "Neurosecretion II. Neurosecretory cells in the nervous system of cockroaches," *J. Comp. Neurol.*, 1941, **74**, 93–108.

———— .. "Hormones in insects," *Hormones* I, 1948, 125–69.

———— .. "Neurosecretion XI. The effects of nerve section on the Intercerebralis-cardiacum-allatum system of the insect *Leucophæa maderæ*," *Biol. Bull.*, 1952, **102**, 261–72.

———— .. "Comparative physiology of invertebrate endocrines," *Ann. Rev. Phys.*, 1953, **15**, 457–72.

———— .. "Neurosecretion in invertebrates," *Publ. Staz. Zool. Napoli*, 1954, Suppl. **24**, 38–40.

Thomsen, E. .. "Functional significance of neurosecretory brain cells and the corpus cardiacum of *Calliphora erythrocephala*," *J. Exp. Biol.*, 1952, **29**, 137–72.

———— .. "Experimental evidence for the transport of secretory material in the axons of the neurosecretory cells of *Calliphora erythrocephala*," *Publ. Staz. Zool. Napoli*, 1954, Suppl. **24**, 48–9.

Thomsen, M. .. "Weissmann's ring and related organs in the larvæ of Diptera," *Dan. Biol. Skr.*, 1951, **6**, 1–32.

Williams, C. M. .. "Morphogenesis and the metamorphosis of insects," *Harvey Lectures*, Ser. **47**, 126–55.


## EXPLANATION OF PLATE

FIG. 1. Photograph of a longitudinal section of late pupa showing two median neurosecretory cells of the median tract in the brain. The axons are very well seen. Fixation Carnoy. Heidenhain's iron hematoxylin. Approx. × 1,000.

FIG. 2. Photograph of the longitudinal section along the margin of the thoracic ganglion of the adult fly showing the anterior and lateral anterior neurosecretory cells. Bouin fixed and stained in Gomori's chrome-hæmatoxylin-phloxin, × 180. Anterior cells to the left.

FIG. 1

FIG. 2

# THE INTIMATE NATURE OF MUSCULAR CONTRACTION

By Sunita Inderjit Singh, M.D. and Inderjit Singh, F.A.Sc.
*(From the Department of Physiology, Medical College, Agra)*

Received October 4, 1954

According to modern theory, denaturation consists of an alteration of the specific internal structure of the protein wherein the closely folded peptide chains unfold. Similarly, the contraction of muscle is supposed to be due to folding of the contractile protein, so relaxation would be due to unfolding of the muscle proteins. These views have been substantiated by experiments showing that some of the agencies, both physical and chemical, which denature proteins, cause active relaxation of heat killed unstriated muscle (Singh and Singh, 1954 *a*, *b*).

In the above papers, it has been supposed, that contraction of unstriated muscle, which does not require energy for its maintenance, is due to the development of linkages between the folded polypeptide chains, thus holding the chains in their folded configuration without the expenditure of energy. In the present research an attempt has been made to test this hypothesis. It has been found that the procedures which destroy those cross linkages abolish the power of the muscle to contract; reintroduction of the cross linkages again enables the muscle to contract and maintain powerful tension or sustain a weight without expenditure of energy.

The qualitative action of certain substances, such as urea, distilled water, acids, alkalies, etc., on heat killed muscle has already been described (Singh and Singh, 1954 *b*); in the present paper their action is described from the quantitative aspect.

## Experimental

The experimental procedure has been as described previously (Singh and Singh, 1954 *a*, *b*). These experiments have been performed on unloaded transverse pieces of the stomach muscle of the frog, *Rana tigrina*; the muscle was killed by gradually raising its temperature to 50° C. and keeping at that temperature for one minute. The effect of various solutions was tested by keeping the unloaded muscle immersed for 24 hours (Singh and Singh, 1949, 1950, 1951). In the present paper, the term "active relaxation" is used for dead muscles, if they lengthened without the

application of any external load. The criteria for active relaxation of living muscle have already been described (Singh and Singh, 1952).

Pieces of muscle were removed from the middle part of the stomach; those from near the cardiac and pyloric ends were rejected, owing to curvature in these places, especially near the pyloric end. In all experiments, control muscles were used. The experimental and control muscles were removed from the adjacent parts of the same stomach. The length of heated muscles was measured before and after heating and after immersion in the experimental solution. If the controls were unheated dying muscles, then the extent of active relaxation was calculated by taking into consideration the initial length of the muscle before heating. This was considered desirable, as it was found that the active relaxation produced by heating, supplemented that produced by various substances and *vice versa*.

## RESULTS

### *Denatured muscle*

In denatured muscle, the bonds which hold the folded polypeptide chains will be ruptured; so the importance of these cross linkages in contraction were elucidated by studying the effect of various substances on muscles, denatured by heating to 50° C.

*Effect of potassium chloride.*—Potassium chloride 0·112 *M*, causes active relaxation of heated and unheated muscles (Table I); the heated muscles have relaxed by 38 per cent. (last column) and the unheated by 22 per cent. (second column). Heating therefore favours relaxation. This is no doubt due to the fact that the process of relaxation is similar to denaturation. This further shows, that heating up to 50° C. could not have caused much change in the interval configuration of the contractile protein molecule, as active relaxation appears to be unimpaired.

Active relaxation which occurs in normal living muscle appears to be similar to denaturation; a few experiments have been described previously in this connection (Singh and Singh, 1954 *b*). To prove this further, it has to be shown that potassium chloride causes relaxation of the contractile mechanism in living muscle and that relaxation produced by potassium chloride is identical with that produced by denaturation.

Experimental evidence favours the view that relaxation produced by potassium chloride is identical with that produced by denaturation. In Table I, the second column shows the effect of potassium chloride on dying unstriated muscles (Singh and Singh, 1949, 1950, 1951); they have relaxed by 22 per cent. The third column shows the effect of heat, which has

TABLE I

*Effect of immersion of heated and unheated, dying frog's stomach muscle in 0·112 M potassium chloride for 24 hours*

| Number of muscle | LENGTH OF MUSCLE AS PER CENT. OF INITIAL LENGTH | | | | |
|---|---|---|---|---|---|
| | Length of control unheated muscle in potassium chloride | Effect of heating experimental muscle to 50° C. | Length of heated muscle in potassium chloride calculated with initial length after heating | Calculated length of heated muscle in potassium chloride based on initial length before heating. Sum of figures in columns 3 and 4 | Observed length of heated muscle in potassium chloride based on initial length before heating |
| 1 | 110 | 125 | 116 | 141 | 145 |
| 2 | 133 | 133 | 104 | 137 | 138 |
| 3 | 130 | 120 | 120 | 140 | 143 |
| 4 | 114 | 109 | 121 | 130 | 132 |
| 5 | 128 | 122 | 123 | 145 | 150 |
| 6 | 116 | 95 | 130 | 125 | 122 |
| Average | 122 | 117 | 119 | 136 | 138 |

produced active relaxation by 17 per cent. The fourth column shows active relaxation of heated muscles by potassium chloride; the average increase in length is 19 per cent. The fifth column shows the combined effect of heat and potassium chloride, as calculated by adding the figures in the third and fourth columns; the average increase in length is 36 per cent. The last column shows the effect of potassium chloride on heated muscles in which the active relaxation has been calculated by taking into consideration the initial length of the muscles before heating; it amounts to 38 per cent., a figure which agrees with the calculated value of 36 per cent. in the fifth column. Further it will be seen in individual experiments, that if heat causes much active relaxation, then potassium chloride causes very little, or if the effect of heat is small, that of potassium chloride is much in evidence. This shows that the effects of heat and potassium chloride are supplementary and therefore suggest that the active relaxation produced by the two agents has more or less a common basis. The third column

shows that heated muscles relax less in potassium chloride, than the unheated ones; the last column, however, suggests the opposite. The latter interpretation appears to be the correct one, because the behaviour of individual heated muscles is not uniform, some relaxing less and the others more than the unheated ones. This view is further supported by the fact that loaded muscles, if heated, always relax more than the unheated ones (Singh and Singh, 1954 *b*).

Potassium chloride appears to produce active relaxation of the contractile mechanism by direct action in living muscle. This is shown by three sets of experiments. Potassium chloride causes active relaxation of dying muscle, in which the muscle may recover its excitability (Singh and Singh, 1949). Increase in osmotic pressure of the saline increases the concentration of potassium inside the fibres (Gokhale and Singh, 1945); increase in osmotic pressure of the saline is known to cause relaxation of unstriated muscle. *Mytilus* muscle rapidly gains potassium if immersed in 0·564 *M* potassium chloride (Singh, 1944). The muscle becomes completely inexcitable, but relaxes and shows marked reduction in viscosity (Singh, 1938 *b*). If the muscle is immersed for 16 hours, it does not recover its excitability, but the deficit in ionic concentration shows that its permeability is intact and hence the muscle is living (Singh, 1944); such a muscle also shows relaxation and marked reduction in viscosity, and the muscle is unable to sustain a weight.

The above experiments suggest, therefore, that in muscular relaxation, which is akin to denaturation, the cross linkages are broken. These cross linkages hold the polypeptide chains together, and thus enable the muscle to exert considerable tension, or sustain a considerable weight without expenditure of energy.

Small concentrations of magnesium chloride antagonise the effect of potassium chloride. Thus the length of 6 heated muscles immersed in 0·112 *M* potassium chloride was 124 $\pm$ 4 per cent. of initial length after heating; in the presence of 0·02 *M* magnesium chloride, it was 111 $\pm$ 5 per cent. Small concentrations of calcium chloride, 0·01 *M*, slightly accentuate the action of potassium chloride. Thus the length of 6 heated muscles in 0·112 *M* potassium chloride was 118 $\pm$ 9 per cent.; in the presence of calcium it was 121 $\pm$ 5 per cent. Calcium has no significant antagonistic action on that of magnesium chloride. Thus the length of 6 muscles in 0·1 *M* potassium chloride and 0·02 *M* magnesium chloride was 116 $\pm$ 4 per cent. in the presence and absence of 0·02 *M* calcium chloride. Ammonium chloride, 0·112 *M*, causes active relaxation of heated muscle by about 20–25 per cent.

*Effect of sodium chloride.*—Sodium chloride causes slight increase in the length of heated muscles, thus the length of 6 heated muscles immersed in 0·112 *M* sodium chloride was 106 ± 7 per cent. of the initial length after heating. The effect however is variable; in one batch there was no increase in length, in some other batches it increased by 19, 16 and 15 per cent. respectively. Small concentrations of magnesium and calcium antagonise the effect of sodium chloride. Potassium chloride also causes greater increase in length in those batches on which sodium chloride is more effective. Unheated dying muscles differ from heated ones in that they contract in sodium chloride; heated muscles never contract.

The effect of sodium chloride is interesting from two points of view:

(1) Unstriated muscle contains more sodium than striated muscle. These experiments suggest that the normal intrinsic tone and viscosity of unstriated muscle is due to its sodium content, as striated muscle does not exhibit such tonus as found in unstriated muscle, and its viscosity is also less. This is also shown by experiments that the active relaxation of heated muscle by potassium chloride is diminished if part of it is replaced by sodium chloride. Increase in sodium content of unstriated muscle results in increase of tone (Singh, 1938 *a*).

(2) It has been shown by Hodgkin and Katz (1949), that sodium enters nerve during excitation. Sodium content of unstriated muscle increases as a result of stimulation (Singh, 1939). It is therefore possible that sodium might be the link between the excitatory and the contractile systems.

The action of other sodium salts, bromide, nitrate and iodide resembles that of sodium chloride, in that they cause slight lengthening of heated muscles. Sodium thiocyanate, 0·112 *M*, causes active relaxation of heated muscles by about 20 per cent. The effect of these anions varies in the following order:

$$Cl = Br < NO_3 < I < SCN < CN$$

Sodium pyrophosphate, 0·06 *M*, causes lengthening by about 20 per cent. Other sodium salts, flouride, phosphate, acetate, citrate, tartarate, sulphate, have no significant action.

Lithium chloride, 0·112 *M*, causes active relaxation of heated muscle by about 23 per cent. The effect of monovalent cations varies in the order

$$Na < Li < NH_4 < K$$

*Effect of sodium cyanide.*—The action of sodium cyanide is interesting, as by its action it has been possible to differentiate two kinds of contractile

mechanisms in unstriated muscle (Singh and Singh, 1951, 1954 *b*); one kind is found in *Mytilus* muscle, and the other in frog's and mammalian unstriated muscle. The length of 6 muscles immersed in 0·112 *M* sodium cyanide was 142 ± 20 per cent. of initial length after heating and 182 ± 50 per cent. of initial length before heating; the length of unheated muscles was 158 ± 51 per cent. As with potassium chloride, heated muscles appear to relax less than the unheated ones, but if the behaviour of individual muscles is seen, then some heated muscles relax less and others more, the action of heat and sodium cyanide being supplementary. Therefore, the initial length before heating has to be taken into consideration; it is then seen that heating favours relaxation.

*Effect of hydrogen ions.*—The effect of potassium chloride in producing active relaxation of unstriated muscle diminishes with increased acidity. Thus the length of 6 heated muscles in 0·112 *M* potassium chloride solutions of pH 5, 7 and 9 (phosphate buffer) was respectively 104 ± 4, 113 ± 6 and 130 ± 9 per cent. of initial length after heating. The hydrogen ions therefore favour the formation of cross linkages or hydrogen bonds. This is supported by the observation that urea disrupts hydrogen bonds and produces marked active relaxation of heated muscle.

*Effect of barium chloride.*—Barium chloride is another interesting agent that acts differentially on the contractile mechanism of *Mytilus* muscle on the one hand and frog's unstriated muscle on the other hand, causing extreme contraction of the former and active relaxation of the latter (Singh and Singh, 1949, 1951). Thus the length of 6 heated frog's muscles in 0·07 *M* barium chloride was 114 ± 5 per cent. of initial length after heating.

Chlorides of calcium, strontium and magnesium have no significant action on the heated frog's muscle.

*Effect of distilled water.*—Distilled water causes active relaxation of heated frog's muscle, but its action differs from that of salts in two respects: (1) It is several times more powerful. (2) The relaxation of heated muscles is less than that of unheated ones, whether the initial length taken into consideration is that before or after heating the muscle. Thus the length of 6 heated muscles was 155 ± 15 per cent. with initial length after heating and 160 ± 16 per cent. with initial length before heating, the length of 6 control unheated muscles being 177 ± 27 per cent. Thus heating diminishes the effect of distilled water, and the point of attack of distilled water differs from that of salts described above. This is in agreement with previous findings, in which the same conclusion was arrived at by a different

technique (Singh and Singh, 1953). In this technique the dying muscle is allowed to relax passively in distilled water or urea solution. After maximal relaxation in 24 hours, potassium chloride produces further relaxation. If the muscle is allowed to relax actively in distilled water or urea solution, potassium chloride will not produce any further relaxation. This shows that urea and potassium chloride act on different linkages. Urea presumably acts on hydrogen bonds, and potassium chloride on salt linkages. These experiments have been confirmed on heat killed muscle, though the dying muscle responds better.

*Effect of urea.*—The action of urea resembles that of distilled water. Thus the length of 6 heated muscles in 0·224 *M* urea was 162 ± 11 per cent. with initial length before heating, and 179 ± 18 per cent. with initial length after heating, the length of 6 unheated muscles being 193 ± 30 per cent.

Effect of thiourea, urethane and formamide resembles that of urea. Thus the length of 6 heated muscles in 0·224 *M* thiourea was 116 ± 9 per cent. with initial length before heating, and 169 ± 11 per cent. with initial length after heating, the length of 6 unheated muscles being 188 ± 18 per cent. The length of 6 heated muscles in 0·224 *M* urethane was 157 ± 10 per cent. with initial length before heating and 164 ± 14 per cent. with initial length after heating, the length of 6 unheated muscles being 198 ± 77 per cent. The length of 6 heated muscles in 0·224 *M* formamide was 157 ± 7 per cent. with initial length before heating and 161 ± 10 per cent. with initial length after heating, the length of 6 unheated muscles being 186 ± 12 per cent. The length of 6 heated muscles in 0·224 *M* acetamide was 132 ± 10 per cent. with initial length before heating and 138 ± 26 per cent. with initial length of 6 unheated muscle being 135 ± 13 per cent.

Effects of other organic substances were tested. Methyl alcohol, ethyl alcohol, up to 40 per cent. in saline, and saline saturated with chloroform, have no significant action on heated muscles. Isotonic solutions of sucrose and glucose cause active relaxation of heated muscles by about 13 and 10 per cent. respectively. The interesting point is that many of the substances that have been mentioned hitherto, cause contraction of unheated muscles but not that of heated ones.

*Effect of hydrochloric acid.*—The action of concentrated solutions of hydrochloric acid will be considered later. Hydrochloric acid, 0·112 *M*, causes active relaxation of heated muscles. Thus the length of 6 heated muscles in acid was 127 ± 23 per cent. with initial length after heating and 135 ± 19 per cent. with initial length before heating, the length of

6 unheated muscles being 129 ± 10 per cent. Hydrochloric acid thus acts like salts.

*Effect of alkalies.*—Alkalies cause active relaxation of heat killed muscle. Thus the length of 6 heated muscles in 0·112 *M* sodium hydroxide was 150 ± 19 per cent. with initial length after heating and 166 ± 30 per cent. with initial length before heating, the length of 6 unheated muscles being 166 ± 37 per cent. The length of 6 heated muscles in 0·112 *M* sodium carbonate was 143 ± 14 per cent. with initial length after heating, and 166 ± 15 per cent. with initial length before heating, the length of 6 unheated muscles being 171 ± 20 per cent. The length of 6 heated muscles in 0·112 *M* sodium bicarbonate was 134 ± 5 per cent. with initial length after heating, and 135 ± 7 per cent. with initial length before heating, the length of 6 unheated muscles being 136 ± 6 per cent. The alkalies, therefore, act like salts.

*Effect of adenosinetriphosphate.*—ATP failed to produce any contraction of heated muscle. This is in accord with the above observations that denatured muscle does not contract.

*Changes in weight.*—Heating up to 50° C. may cause either slight increase or decrease in weight, but there is no correlation between change in weight and change in length. Thus in 6 experiments, the first muscle contracted by 11 per cent., but the weight increased by 4 per cent. The second muscle lengthened by 65 per cent., but the weight decreased by 3 per cent. The third muscle lengthened by 14 per cent., and decreased in weight by 3 per cent. The fourth muscle increased in length by 10 per cent., but decreased in weight by 2 per cent. The fifth and sixth muscles did not change length, but decreased in weight by 3 per cent. Thus there is no significant change in weight, the changes that were recorded can fall within the experimental error of weighing a wet muscle. If the muscle is heated to 60° C., then it may contract or relax, but the weight always decreases by about 10 per cent.

*Coagulated muscle*

The chief finding about denatured muscle is that it has lost the power of contraction. In denatured muscle, the cross linkages between the folded polypeptide chains are ruptured, so it appears that these cross linkages are necessary for contraction. This view would receive support if it was possible to reintroduce these cross linkages and then study the behaviour of muscle. One view of coagulation of proteins is that it is due to the formation of intermolecular and intramolecular salt bridges

between ionic groups which are displaced to the surface of the particle through the unfolding of the peptide chains (Haurowitz, 1950). If this view is correct then coagulation would reintroduce these cross linkages, but they would not be the same as before denaturation.

Though most substances which cause contraction of the dying muscle, fail to cause contraction of heated muscle, it has been found that agencies that cause coagulation of proteins produce powerful contraction of heated muscle. The effect of heat and some protein coagulants has therefore been studied.

Heating up to 50° C. causes relaxation of loaded muscle (Fig. 1). Further heating up to 60° C. causes powerful contraction of dog's and frog's

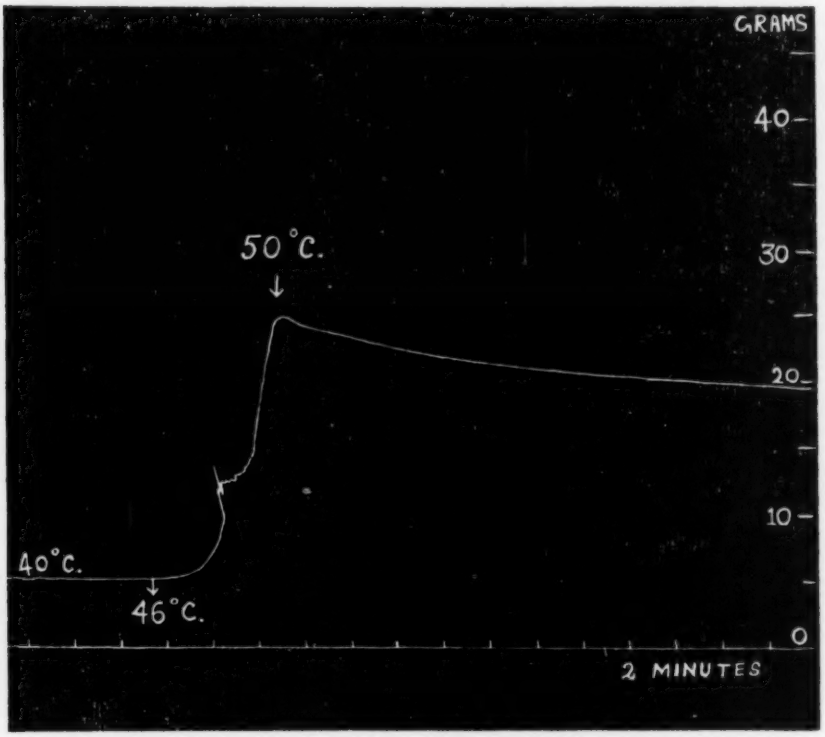



FIG. 1. Dog's stomach muscle. Effect of heating.

stomach muscle. A still further contraction is caused at 70° C., which increases up to 90° C. The muscle exerts considerable tension and lifts

a weight indefinitely. At such high temperatures, collagen is likely to liquefy, hence the contraction is due to the muscle proteins. The effects of heat are slightly different in individual muscles. Some muscles contract at 50° C. and relax at 60° C. and contract after 70° C. up to 95° C. (Fig. 2).

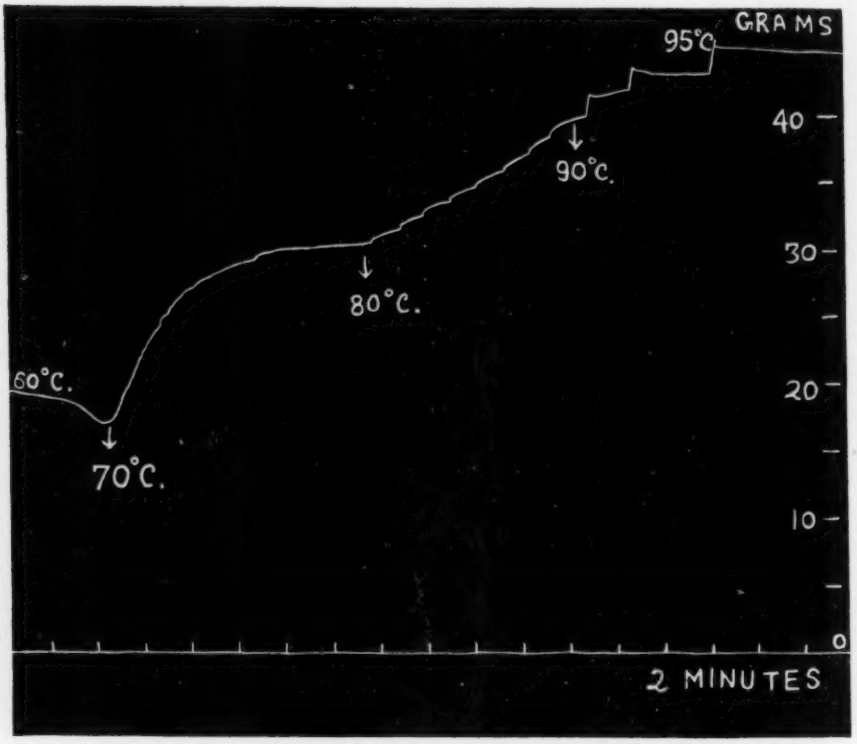



FIG. 2. Dog's stomach muscle. Effect of heating.

Normal hydrochloric acid which coagulates the muscle, caused contraction of 6 heated unloaded frog's stomach muscle by about 15 per cent. The action was reversed by deci-normal sodium hydroxide (Singh and Singh, 1950). Loaded muscles only relax in hydrochloric acid.

Mercuric chloride causes coagulation of muscle. It may cause shortening or active relaxation of heated unloaded frog's stomach muscle. The latter is no doubt due to denaturation. Thus in 6 experiments, mercuric chloride, 1 in $10^3$ in saline, caused contraction of three muscles by about 20–25 per cent. and active relaxation of three muscles by 15–20 per cent. In another 6 experiments, mercuric chloride, 1 in $10^4$, caused contraction

all the muscles. Dying muscles are similarly affected; out of 12 experiments, contraction occurred in 9 muscles. Mercuric chloride, 1 in $10^5$, has no significant action. Loaded frog's muscles only relax in mercuric chloride, but dog's stomach muscle may show a feeble contraction in 1–5 per cent. solution.

Zinc sulphate causes contraction of heated muscle. Thus in 6 experiments, 0·112 *M* zinc sulphate caused contraction of 6 muscles by about 5–10 per cent. Trichloracetic acid, 0·112 *M*, caused relaxation by about 16 per cent. Sodium tungstate produced relaxation. Loaded muscles only relax in these solutions.

*Reactions of coagulated muscle.*—The properties of coagulated muscle were studied to see how far the cross linkages in normal muscle differ from those in coagulated muscle. Frog's stomach muscle was coagulated by heating in saline at 70° C. for 10 minutes. Urea, urethane, formamide, 0·224 *M* each, caused slight relaxation of unloaded muscle by about 5 per cent. Distilled water and potassium chloride had similar action. Sodium hydroxide, 0·112 *M*, at first caused relaxation and then shortening; this last action is opposite that on dying or denatured muscle. Sodium carbonate, 0·112 *M*, caused relaxation by 10–20 per cent. Sodium cyanide, 0·112 *M*, caused relaxation by about 20–25 per cent. Hydrochloric acid, 0·112 *M*, caused slight relaxation.

The above experiments show that the cross linkages in coagulated muscle do resemble those in normal muscle, but to a small extent only. The contraction of coagulated muscle strongly supports the view that cross linkages between the folded polypeptide chains are necessary for contraction.

*Heat effects on living muscles.*—If unstriated muscle is heated beyond 40° C., it may either relax actively or passively or contract before it dies near about 48° C.; frog's stomach muscle relaxes and dog's stomach muscle contracts. This effect of heat is on the contractile mechanism, as the relaxed muscle is inexcitable, and the contraction is not affected by cyanide or asphyxia; on cooling the muscle recovers. Active relaxation continues if the muscle is heated to about 60° C. The active relaxation of living muscle passes imperceptibly into that of dead muscle. This suggests that as with urea, distilled water and potassium chloride, heat produces denaturation or a very similar process in living muscle and this is the normal mode of relaxation. Denaturation is known to be reversible in isolated proteins.

Similarly, the heat contraction in living muscle passes imperceptibly into that of dead muscle, so that there is some relation between one kind of tone and coagulation; this relationship is probably the development of cross linkages in both. This is further shown by the fact that the heat contraction occurs in those muscles which show normally much tone, such as dog's stomach muscle. Under suitable conditions the heat contraction may occur at 30° C. and this contraction and that which occurs at 40° C. are identical with the normal tonic contraction which we have previously designated as alactic tone (Singh and Singh, 1949 *b*). The heat contracted muscle relaxes actively at 50° C.

## DISCUSSION

Denaturation appears to cause both active and passive relaxation of the contractile mechanism of muscle. This relaxation is not accompanied by swelling of muscle, and so it differs from actomyosin reactions, in which contraction appears to be syneresis. Denaturation is very closely allied to the relaxation of living muscle, as some of these substances such as distilled water, urea, potassium chloride produce active relaxation of the contractile mechanism in living muscle; this is also shown by the fact that sodium cyanide, which abolishes the active relaxation through the excitatory mechanism, has no effect on their action.

The cross linkages, which hold the folded polypeptide chains together, are of two kinds: one kind are attacked by salts and the other kind by urea group of substances. Cross linkages are necessary for the muscle to sustain tension without expenditure of energy; they are also necessary for contraction as denatured muscle does not contract at all. One kind of cross linkages require energy for their maintenance and these are presumably attacked by salts; they are probably necessary for contraction which requires energy. The other kind of cross linkages are permanent, and thus hold the polypeptide chains without expenditure of energy; these are attacked by urea (Singh and Singh, 1954) and are presumably responsible for tonus which does not require energy.

Coagulated muscle is an example of muscle in tonic contraction. The intramolecular and intermolecular bridges hold the folded polypeptide chains together, as they presumably do in normal tonus. These attractions and repulsions are electrical in nature, so that movement of the muscle will be facilitated in one direction and retarded in the other; thus the catch mechanism of unstriated muscle is explained.

There appears to be some relationship between the cross linkages of coagulated muscle and those of muscle in tonic contraction. Thus dog's

stomach muscle which shows greater tone than frog's stomach muscle, also contracts more strongly as a result of heating. Mercuric chloride causes contraction of heated dog's stomach muscle, but not that of frog's muscle. This shows that the cross linkages as a result of coagulation are more easily formed in those muscles which show greater tone, or in which tonic cross linkages form more easily.

Loaded and unloaded muscles may differ in their responses. Some substances which cause contraction of the contractile mechanism of unloaded muscle, cause relaxation of loaded muscle. This suggests that prior to contraction, there is some loosening of the bonds, so that the muscle relaxes if loaded. This causes strategic points to move too far apart for active forces to be effective. Such a phenomenon also takes place in living muscle. Thus the muscle may be unable to lift weight, but if the lever is lifted with the hand and the muscle allowed to shorten, then the muscle remains contracted when the hand is removed. Similarly, if the muscle is forcibly stretched, then it may be unable to recover its original length on release and remains permanently extended. This experiment suggests that in unstriated muscle some of the attractive forces are a permanent feature.

Sodium is supposed to enter the muscle during excitation, but it is not likely to act as a link between the excitatory and the contractile mechanisms. This is shown by the fact that substances which increase the permeability of *Mytilus* muscle to sodium, decrease the phasic response to electric current. Thus calcium decreases the permeability to sodium, but increases the phasic contraction. The anions increase the permeability to sodium in the order

$$Cl < Br < NO_3 < I < SCN < CN$$

and they also increase the phasic response in the inverse order. But these substances which increase the permeability to sodium, though they decrease the phasic response, increase the subsequent tonic contraction or slow relaxation in the same order. In unstriated muscle, therefore, the entry of sodium is responsible for the slow relaxation which follows a phasic contraction. Potassium takes part in the phasic contraction, as increase in its concentration inside the fibres results in augmentation of the phasic contraction but decrease of tone; this suggests that potassium replaces sodium from the contractile mechanism and *vice versa*.

There is both chemical and physiological evidence for the entrance of ions into the muscle fibres during excitation. Chemical analyses have shown that unstriated muscle of *Mytilus* gains sodium during stimulation. The

same muscle gains sodium in the absence of calcium. The gain of ions by the muscle is increased by blotting. The physiological evidence is as follows: If *Mytilus* muscle is immersed in saline containing barium ions, it passes into a tonic contraction. At other times, especially in summer, the muscle may remain quiescent without any apparent effect; barium ions are present in the saline in both instances, and yet the muscle remains quiescent in one instance and contracts in the other. The quiescent muscle can be made to produce a typical barium contraction by certain methods, such as passage of electric current for a short period insufficient to cause a contraction or produce only a twitch, sudden heating or cooling, sudden stretch or release, stroking the surface with a hair brush or a blotting paper, vigorous aeration with air or an indifferent gas such as nitrogen or hydrogen, removal of calcium and addition of drugs like adrenaline, acetylcholine, caffeine; these drugs by themselves may or may not produce a contraction.

The above effects appear to be unrelated, but there must be a common effect produced by them. The effect produced by removal of calcium suggests increase of permeability and entrance of barium into the muscle. Blotting also, as shown by chemical analyses, increases the gain of ions by the muscle. The above argument regarding barium applies to other ions in the saline, such as sodium and potassium. The permeability of *Mytilus* muscle to cations varies in the following order:

$$Li < Na < NH_4 < K$$

They also retard relaxation and produce tonic contraction in the same order.

Experiments have shown that though the urea group of substances and potassium chloride, both produce active relaxation of heated muscle, they act on different points of the contractile mechanism. There are, therefore, two distinct mechanisms of relaxation. In dying muscle, which is a more physiological preparation than heated muscle, urea group produce active relaxation and potassium chloride mostly passive relaxation, though the latter produces also a little active relaxation. Active relaxation is therefore due to dissolution of hydrogen bonds and passive relaxation is due to dissolution of salt linkages. As potassium chloride causes relaxation of the contractile mechanism of practically all kinds of unstriated muscle tested so far (*Mytilus* muscle, frog's stomach muscle, dog's stomach muscle), one kind of tonus is due to the formation of salt linkages; tonus in *Mytilus* muscle is therefore due to some modification of the salt linkages. The other kind of tonus is due to hydrogen bonds.

## Summary and Conclusions

1. The responses of denatured and heat coagulated unstriated muscle have been studied. In denatured muscle, the cross linkages are destroyed and they are reintroduced by coagulation. Thus the importance of cross linkages in contraction of muscle have been elucidated.

2. Heat denatured muscle is actively relaxed by two groups of substances, salts and urea group; these act on different points of the contractile mechanism.

3. Potassium chloride causes relaxation of the contractile mechanism; sodium chloride has the opposite effect. Sodium of unstriated muscle is therefore considered to be responsible for intrinsic tone viscosity of unstriated muscle.

4. The action of potassium chloride and heat in causing active relaxation of unstriated muscle are supplementary.

5. Small concentrations of magnesium chloride antagonise the action of potassium and sodium chloride. Calcium increases the action of potassium, but opposes that of sodium.

6. The cations cause active relaxation of denatured muscle in the order

$$Na < Li < NH_4 < K$$

7. The anions cause active relaxation of denatured muscle in the order

$$Cl = Br < NO_3 < I < SCN < CN$$

8. Hydrogen ions antagonise the action of potassium chloride.

9. Barium chloride causes active relaxation of denatured muscle; calcium, strontium and magnesium chlorides have no significant action.

10. Distilled water, urea, thiourea, urethane, formamide cause active relaxation of denatured muscle which differs from that produced by salts. Acetamide acts like salts.

11. Acids and alkalies cause active relaxation like that produced by salts.

12. Adenosinetriphosphate does not cause contraction of denatured muscle.

13. There is no significant change in weight during active relaxation produced by denaturation.

14. The relaxation produced by heat, therefore, differ from actomysin reactions, and evidence is produced to show that it is closely akin to relaxation occurring normally in living muscle.

15. Denatured muscle is made to contract by protein coagulants, such as heating, hydrochloric acid, mercuric chloride, zinc sulphate, otherwise denatured muscle has lost the power of contraction.

16. Effect of various substances on coagulated muscle has been described.

17. It is concluded that cross linkages are necessary for contraction. One kind hold the folded polypeptide chains together, and thus account for contraction of unstriaetd muscle not requiring energy.

## REFERENCES


Gokhale, S. K. and Singh, I. .. *Proc. Ind. Acad. Sci.*, 1945, **21**, 202.

Haurowitz, F. .. *Chemistry and Biology of Protein*, Academic Press, New York, 1950.

Hodgkin, A. L. and Katz, B. .. *J. Physiol.*, **108**, 37,

Singh, I. .. *Ibid.*, 1938*a*, **91**, 398.

——————— .. *Ibid.*, 1938*b*, **92**, 232.

——————— .. *Ibid.*, 1939, **96**, 1.

——————— .. *Proc. Ind. Acad. Sci.*, 1944, **20**, 209.

Singh, S. I. and Singh, I. .. *Ibid.*, 1949, **30**, 270.

——————— .. *Ibid.*, 1949 *b*, **30**, 263.

——————— .. *Ibid.*. 1950, **32**, 12.

——————— .. *Ibid.*, 1951, **33**, 165.

——————— .. *Ibid.*, 1953, **37**, 188.

——————— .. *Ibid.*, 1954 *b*.

——————— .. *Curr. Sci.*, 1952, **21**, 283.

——————— .. *Ibid.*, 1954*a*, **23**, 126.


1509–54 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

# BACTERIOLOGY OF OFF-SHORE SEA-WATER OF THE WEST COAST *

By R. Venkataraman and A. Sreenivasan
(*Fisheries Technological Station, Kozhikode*)

Received July 14, 1954
(Communicated by Prof. P. S. Sarma)

In a recent communication Velankar (1954) has given an account of the bacteria in the inshore environment of the East Coast of India and Venkataraman and Sreenivasan (in Press) have described the flora of inshore sea-water of the West Coast. We have presented here the bacterial flora of Off-shore waters of the West Coast—off Tellicherry and off Calicut.

## Materials and Methods

A sample was collected 25 miles off Tellicherry and the other 16 miles off Calicut. Plates were poured on sea-water agar as well as on fresh-water agar and also on VRB agar for coliforms. 10 c.c. quantities were also inoculated into double strength lactose broth for detection of coliforms (presumptive test). Presence of denitrifiers and urea fermenters was also tested in Calicut water samples.

## Results

*Coliforms* were not present in either of the samples of water. Even enrichment in lactose broth did not indicate any positive presumptives.

*Denitrifiers* were shown to be present in 1 c.c. quantities of sea-water. Of the 95 strains studied, as many as 13 were denitrifiers, producing gaseous nitrogen from nitrates. Occurrence of such large numbers of denitrifiers is interesting since Waksman *et al.* (1933 *a*) have stated that they were not present in the surface-waters and hence do not play any part in the loss of nitrogen from the sea. However if we consider the large numbers that occur, there appears to be a chance of nitrogen being lost and probably this is the reason for the poor nitrate content of surface-waters of the tropics which have high temperatures. Of these 13 strains nine were 'marine' species originally isolated from sea-water agar and failing to grow in fresh-water media. Two were from fresh-water agar plates and grew equally well on sea-water as well as on fresh-water media. There was

* Published with the permission of the Government of Madras.

practically no difference in their morphological, cultural and biochemical characteristics but notable differences were found in their amino acid utilisation. The remaining two strains were isolated from sea-water agar plates incubated at 4° C. for 4 months and produced greenish fluorescent soluble pigment. These failed to grow at 37° C. but grew slowly at room temperature of 28–30° C. They also grew well in fresh-water media and they appear to be identical with *Ps. denitrificans.* A detailed account of the denitrifiers is given elsewhere.

*Psychrophiles.*—When 1 c.c. of sea-water was inoculated into sea-water agar plates and incubated at 4° C, only three colonies developed after 4 months. This, in contrast to the 106 mesophiles per c.c. on the same agar, is a poor number. Two of these were the fluorescent *Pseudomonas* mentioned above and one was a *Micrococcus.*

*Bacterial flora.*—Ninety-five strains were isolated from Calicut sea-water and their morphological and cultural characteristics studied. 72 of them were further studied in detail for their biochemical properties. In general pleomorphism appeared to be a common feature and two of the *Bacillus* species were involution forms. The pattern of bacterial flora is the same as described in our earlier work on inshore sea-water and in mackerels. There were numerous aerobic spore-forming *Bacillus* and of these, as many as 10 were distinctly chromogenic, a fact noted in our earlier work. These rose-red *Bacillus* thus seem to be part of the natural bacterial flora of the sea. *Achromobacter* were also found in large numbers and seven of them produced indole. The next important group was *Pseudomonas* and all the 13 were denitrifiers but only two of them produced greenish fluorescent pigment. *Micrococci* were the next numerically large group, *Flavobacter* following up. *Sarcina, Vibrio* and *Bacterium* were also encountered in very small numbers. The results are tabulated in Table I.

Yellow was the dominant colour, 16 of the organisms belonging to *Flavobacterium, Micrococcus, Sarcina* and *Bacillus* having this colour. Red and rose shade was next evident, with a large number of *Bacillus* developing this colour as well as fewer number of *Micrococcus* and a *Bacterium.* Two greenish *Pseudomonas,* one yellowish-orange *Bacillus* and one brownish-black *Bacillus* were also noted.

It could be seen that none of the *Pseudomonas,* only one *Micrococcus,* two *Flavobacter* and four *Achromobacter* were gelatinolytic. They were much less active in digesting casein of milk. On the contrary 25 of the *Bacillus* liquefied gelatin, and 17 digested casein. These were also saccharolytic to a great extent as against very negligible fermentative activity

TABLE I

*Bacterial flora of off-shore sea-water (Calicut) and their biochemical properties*

| | | Gelatin liquefiers | Milk peptonizers | Milk acid coagulated | Fermentation of Dextrose | Hydrolysis of starch | Chromogenesis | Nitrate reducers |
|---|---|---|---|---|---|---|---|---|
| *Achromobacter* | 8 | 4 | 1 | .. | 2 | 1 | .. | 6 |
| *Flavobacterium* | 7 | 2 | 1 | .. | 1 | 2 | 7 | 3 |
| *Micrococcus* .. | 10 | 1 | 1 | 1 | 5 | 3 | 8 | 8 |
| *Sarcina* .. | 3 | 2 | 1 | 1 | 1 | .. | 3 | 1 |
| *Bacillus* .. | 29 | 25 | 17 | 3 | 21 | 5 | 10 | 11 |
| *Pseudomonas* .. | 13 | .. | 2 | .. | 2 | 2 | 2 | 13 (Denitrifiers) |
| *Vibrio* .. | 1 | 1 | .. | .. | .. | 1 | .. | 1 |
| *Bacterium* .. | 1 | 1 | .. | .. | 1 | .. | 1 | .. |
| Total .. | 72 | 36 | 23 | 5 | 33 | 14 | 31 | 43 |

of the *Flavobacter*, *Achromobacter* and *Pseudomonas*. *Micrococci* were slightly more active in fermenting sugars.

*Marine species*.—Nine *Pseudomonas* (*Ps. marinodenitrificans* n.sp.), four *Achromobacter* and one *Flavobacterium* failed to grow in fresh-water media and may be considered to be 'marine' according to ZoBell (1946). All of them were gram negative, non-spore-forming rods which were actively motile. They exhibited very poor saccharolytic and proteolytic tendencies as noted in the case of 'marine' bacteria from mackerels (Venkataraman and Sreenivasan, in Press). None of the *Bacillus*, *Micrococcus*, *Sarcina* or *Bacterium* were 'marine' forms in the above sense. Marine flora in general are characterised by the presence of only a few types, without much diversity.

A study of 32 cultures isolated from off-shore sea-water off Tellicherry showed the presence of *Bacillus*, *Micrococcus*, *Sarcina*, *Flavobacterium*, *Achromobacter*, *Bacterium*, *Corynebacterium* and *Alcaligenes*. Here also yellow was the dominant colour but there were also as many as seven rose-red *Bacillus* species similar to the ones found in Calicut sea-water. These rose-red spore-forming *Bacillus*, thus have been repeatedly obtained from marine environment over a wide area examined. In this sample also

the *Flavobacter* were inert, never acting on sugars or on proteins and thus confirm their unimportant role in fish spoilage (Castell and Mapplebeck, 1952). The *Bacillus* on the other hand showed rapid proteolytic action on gelatin and casein, and equally active saccharolytic powers. The species commonly noted were *Achromobacter superficiale*, *Flavobacterium marinum*, *Fl. solare*, *Fl. flavus*, *Bacterium zopfi*, *Sarcina flava*, *Micrococcus flavus* and the rose-red *Bacillus* belonging to the *subtilis* group.

Nitrate reduction by sea-water bacteria seems to be very common. About 50% of the organisms from Calicut and Tellicherry sea-water reduced nitrates. Nitrate reduction was marked in species of *Micrococcus*, *Pseudomonas* and *Corynebacterium*. In the case of *Bacillus* in many cases there was an inverse correlation between gelatin liquefaction and nitrate reduction. In *Micrococci* and *Pseudomonas* also there was a similar trend, more of them reduced nitrates but did not liquefy gelatin. Of the *Pseudomonas* all the 13 produced free nitrogen from nitrates.

Starch hydrolysis was not uncommon and quite a few of the species possessed amylase. Very few species were acido-proteolytic in milk.

## DISCUSSION

The bacterial flora of the off-shore sea-water of the West Coast is not a varied one but consists of a limited number of types, which are characteristic. As in the case of inshore sea-water and of marine fish (Venkataraman and Sreenivasan, *loc. cit.*) the dominant groups were *Achromobacter*, *Micrococcus*, *Pseudomonas* (non-chromogenic), *Bacillus* and *Flavobacter* with *Sarcina*, *Bacterium* and *Corynebacterium* occurring in smaller numbers. The absence of coliforms only confirms the inability of this group to establish itself in marine environment (ZoBell, 1946; Ketchum *et al.*, 1949; Vaccaro *et al.*, 1950). Though Waksman *et al.* (1933) consider the occurrence of bacteria reducing nitrates to free nitrogen in open sea to be limited, we found that 13 of the 95 cultures isolated from off-shore water, 16 miles off Calicut were denitrifiers and 9 of them requiring sea-water for growth. Though the organic matter in surface-water may be poor, it is possible for these organisms to obtain their requirements of nutrients as commensals on plankton.

Though fluorescent *Pseudomonas* were not encountered by us in earlier studies, two were isolated from Calicut sea-water, and these were from plates incubated at 4° C. for 4 months. Only three colonies appeared on this plate which clearly shows the poverty of psychrophilic types in tropical waters. These two fluorescent rods in fact did not grow at

37° C. but grew slowly at 28–30° C. Though the non-fluorescent *Pseudomonas* were 'marine' forms, the two fluorescent ones grew in fresh-water media with equal facility. Since we isolated greenish fluorescent *Pseudomonas* more frequently from green mussels near shore (unpublished results), it should be considered that the fluorescent rods are not 'marine' types.

Repeated isolation of the chromogenic (rose-red) *Bacillus*, from both the waters, *viz.*, from Tellicherry and Calicut, and also from other samples reported earlier, emphasizes the fact that these are not adventitious forms but are the types which have established themselves well in the marine environment. Wood (1953) also reported the occurrence of red *Bacillus* in Australian sea-water.

When we consider that all the *Bacillus*, *Micrococci* and *Bacterium* so far studied grow well in fresh-water as well as in sea-water media but that some of the *Achromobacter*, *Flavobacterium* and *Pseudomonas* fail to grow in the absence of sea-water in media, it appears probable that there is a specific 'marine' flora as suggested by ZoBell (1946). These were all gram negative, actively motile, non-spore-forming rods with very poor fermentative properties as emphasized by ZoBell (*loc. cit.*). However as suggested by Wood (1950, 1953) there might be a gradation from the soil types through estuarine to typically marine flora. This is in keeping with the views of Burke (1934) and Burke and Baird (1931), who suggested that there is an exchange of marine and fresh-water forms near the sea and that some of the fresh-water forms maintain themselves in sea-water without their physiological activities being affected.

## Summary and Conclusions

The bacterial flora of the off-shore sea-water of Tellicherry and Calicut was comprised of a limited number of types—*Achromobacter*, *Bacillus*, *Micrococcus*, *Flavobacterium*, *Pseudomonas*, *Bacterium*, *Corynebacterium* and *Sarcina*. All the *Pseudomonas* were denitrifiers, including the two fluorescent ones. There were only very few psychrophiles but a large number of thermoduric *Bacillus* species. For preservation use of cold is indicated because of the poverty of psychrophiles and abundance of mesophilic forms.

## References

Burke, V. .. "Interchange of Bacteria between fresh-water and the sea," *J. Bact.*, 1934, **27**, 201–05.

—— and Baird, L. .. "Fate of fresh-water bacteria in the sea," *ibid.*, 1931, **21**, 287.

Castell, C. H. and Mapplebeck, E. G. "The importance of Flavobacterium in fish spoilage," *Jour. Fish Res. Bd.* (Can), 1952, **9**, 148–56.

Ketchum, B. H., Carey, C. L. and Briggs, M. P. "Preliminary studies, on the viability and dispersal of coliform bacteria in the sea," *Amer. Assoc. Adv. Sci.*, 1949, **40**, 64–73.

Vaccaro, R. F., Briggs, M. P., Carey, C. L. and Ketchum, B. H. "Viability of *Eschetichia coli* in sea water," *Amer. J. Publ. Health*, 1950, **40**, 1257–266.

Venkataraman, R. and Sreenivasan, A. (in Press in *Proc. Nat. Inst. Sci.*).

Velankar, N. K. .. "Bacterial flora of an inshore environment," *Proc.* 41*st Indian Sci. Congr.*, Pt. III. 171 (*Abstr.*), 1954.

Waksman, S. A., Reuszer, H. W., Carey, C. L., Hotchkiss, M. and Renn, C. E. "Studies on the Biology and Chemistry of the gulf of Maine, III.—Bacteriological investigations of the sea-water and marine bottoms," *Biol. Bull.*, 1933*a*, **64**, 183–205.

——, Hotchkiss, M. and Carey, C. L. "Marine Bacteria and their role in the cycle of life in the sea, II. Bacteria concerned in the cycle of nitrogen in the sea," *Biol. Bull.*, 1933*b*, **65**, 137–67.

Wood, E. J. F. .. "Bacteria in marine environment," *Indo-Pacific Fish. Counc., Proc.*, 1950, 69.

——— .. "Heterotrophic bacteria in marine environment of Eastern Australia," *Austr. J. Mar. Freshw. Res.*, 1953, **4**, 160–200.

ZoBell, C. E. .. *Marine Microbiology*, Chronica Botanica Co., Waltham, Mass, 1946, pp. 1–240.

# MICROCHROMATOGRAPHY

## Part II. Detection of Trace Elements in Biological Media

By K. Lakshminarayanan
(*University Botany Laboratory, Madras-5*)

Received December 3, 1954
(Communicated by Prof. T. S. Sadasivan, F.A.SC.)

### Introduction

Since the advent of partition chromatography considerable attention has been directed towards the separation and identification of inorganic cations on paper strips and the interesting review by Pollard and McOmie (1951) points to the potentialities of the technique as a micro-analytical tool. But the usual ascending and descending chromatographic techniques employed in these studies are beset with the main difficulty due to the diffusion and overlapping of adjacent cationic bands in mixtures. Airan (1952) and Airan and Barnabas (1953) have indicated the advantages of paper disk chromatography for the separation of inorganic cations. Employing the detachable wick technique developed by Giri and Rao (1952 *a*, *b*) they have reported the separation of copper, nickel and cobalt in the order of 100 $\mu$g. The technique of microchromatography developed by the author (Lakshminarayanan, 1954 *b*) has been successfully adapted for the detection of trace elements of biological importance in the order of 0·15 to 0·2 $\mu$g. in mixtures and in biological media and reported hereunder.

### Methods and Materials

The technique employed was identical with the one reported earlier (Lakshminarayanan, 1954 *b*). The heavy metals were divided into three groups for purposes of separation and identification: (I) Cu, Ni and Co; (II) Fe, Mn and Al; (III) B, Mo and Zn. The salts used throughout were of analar grade and distilled water used was ion-free (from all-glass Pyrex distiller). The following salts of the cations were studied: $CuCl_2.2H_2O$; $NiSO_4.7H_2O$; $CoCl_2.6H_2O$; $FeCl_3.6H_2O$; $MnCl_2.2H_2O$; $AlCl_3.6H_2O$; and $(NH_4)_2SO_4.Al_2(SO_4)_3.24H_2O$; $Na_2B_4O_7.10H_2O$ (neutralized with HCl to pH 6·5 to 7·0); $(NH_4)_6Mo_7O_{24}.4H_2O$ (neutralized with $HNO_3$ to pH 6·5 to 7·0) and $ZnSO_4.7H_2O$. The salts were also incorporated in

Richard's medium (diluted 1 : 2) of composition (Sucrose—50 g.; $KNO_3$—10 g.; $KH_2PO_4$—5 g.; $MgSO_4.7H_2O$–2·5 g.; Distilled water–1 litre). The concentration of the cations in mixtures was approximately 0·15 to 0·2 $\mu$g. of cation in 0·25 $\mu$l. spotted in the smallest area possible. The solvent mixtures employed were: (i) N-butanol-acetic acid-water (4 : 1 : 5); (ii) Aqueous collidine (2 : 4 : 6 Collidine); and (iii) Aqueous lutidine (2 : 4/2 : 5 Lutidine). The mixtures were separated in solvent (i) and confirmed by separation in solvents (ii) and (iii). Filter paper used: Whatman No. 1. Diameter of the solvent boundary: 2·3 cm. Time of irrigation was maintained at 40 to 45 min. throughout. At the end of the irrigation the filter papers were air-dried, sprayed with the detecting agents, examined and $R_f$ values determined as described earlier (Lakshminarayanan, 1954 *h*).

*Spray reagents employed for detection of cations.*—Group I was detected by spraying with rubeanic acid (0·1% in acetone) and the colour of the bands was intensified by exposure to ammonia vapour for 10 sec. Groups II and III were detected by spraying with a solution of 8-Hydroxyquinoline in absolute alcohol (0·2%), air-dried and examined under ultra-violet lamp (3560 Å). The following simple procedure was however found to be better than spraying with 8-Hydroxyquinoline: The dried chromatograms were left in a descicator containing approx. 0·5% solution of 8-Hydroxyquinoline in absolute alcohol for three hours when the reagent diffused and got uniformly impregnated on the filter paper. Confirmatory reagents employed were: (i) Sodium diethyl dithio carbamate (0·05% in acetone); (ii) Ferron (7-iodo 8-hydroxyquinoline 5-sulphonate) 0·1% solution in alcohol; (iii) Acridine hydrochloride: 0·1% in alcohol.

## RESULTS AND DISCUSSION

Table I gives the reagents used to identify the cations in the micro-chromatograms illustrated in Plate IV (Figs. 1 to 3). Rubeanic acid and 8-Hydroxyquinoline have been found useful for detection of the individual cations of Groups I and (II and III) respectively. The others were employed for confirmation. $H_2S$ gas which has been reported by other workers (Airan and Barnabas, 1953; Lacourt *et al.*, 1949) for detection of Cu, Ni and Co bands was not found to be sufficiently sensitive at the concentrations of the cations employed ; besides higher concentrations of cations generally caused diffusion of the bands.

Table II gives the $R_f$ values of the cations in the three solvent mixtures: N-butanol-acetic acid-water (4 : 1 : 5); aqueous collidine; and aqueous lutidine. The values represent the mean of 6 chromatograms to an accuracy of 3%.

TABLE I

*Reagents used to identify the cations in the microchromatograms*

| No. | Reagents | Cations | Nature and colour of the bands |
|---|---|---|---|
| 1 | Rubeanic acid (followed by exposure to $NH_3$ vapour) | Cu″ | Olive green, intensifying on exposure to $NH_3$ vapour |
| | | Co″ | Yellow, turning brown on exposure to $NH_3$ vapour |
| | | Ni″ | Blue, turning purple on exposure to $NH_3$ vapour |
| 2 | 8-Hydroxyquinoline (Chromatograms observed in ultra-violet 3560 Å) | Background | Light greenish-yellow fluorescence due to the reagent |
| | | Fe‴ | Pink, opaque |
| | | Mn″ | Pink, opaque, slightly diffused |
| | | Al‴ | Greenish-yellow, fluorescent |
| | | B‴ | Deep yellow, fluorescent |
| | | Mo‴ | Pink, opaque |
| | | Zn″ | Yellow, fluorescent (losing fluorescence on continued exposure) |
| 3 | Acridine hydrochloride (Chromatograms observed in U.V. 3560 Å) | Background | Light bluish fluorescence due to the reagent |
| | | Fe‴ | Dark green, opaque |
| | | Cu″ | Dark green, opaque |
| | | Co″ | Light green |
| | | Ni″ | Light green |
| 4 | Sodium diethyl dithio carbamate | Cu″ | Dark brown |
| | | Fe″ | Light brown |
| 5 | Ferron .. .. | Fe‴ | Dark green |

It would be evident from Table II that the first group of elements Cu, Ni and Co could very easily be separated and identified in *n*-butanol-acetic acid-water in view of the wide differences in their $R_f$ values in this mixture. The order of the cations was reversed in the other two solvent mixtures. The second group of elements Fe, Mn and Al could also be effectively separated in *n*-butanol mixture. Though Mn and Al have close $R_f$ values the bands presented marked contrast with 8-Hydroxy quinoline, the former pink and opaque and the latter fluorescent yellow. Iron occupied the outermost position in all the three solvent mixtures,

TABLE II

*$R_f$ values of the cations in different solvent mixtures*

| No. | Cations | Solvent Mixtures | | |
|---|---|---|---|---|
| | | N-butanol-acetic acid-water 4 : 1 : 5 | Aq. Collidine | Aq. Lutidine |
| 1 | Copper .. | 0·631 | 0·277 | 0·613 |
| 2 | Cobalt .. | 0·373 | 0·231 | 0·511 |
| 3 | Nickel .. | 0·258 | 0·427 | 0·285 |
| 4 | Iron .. | 0·464 | 0·435 | 0·498 |
| 5 | Manganese .. | 0·222 | 0·259 | 0·237 |
| 6 | Aluminium .. | 0·191 | * | * |
| 7 | Boron .. | 0·479 | 0·217 | 0·370 |
| 8 | Molybdenum .. | 0·291 | 0·279 | 0·232 |
| 9 | Zinc .. | 0·202 | * | * |

* These ions do not move in the solvent mixtures.

Al and Zn ions did not move in aqueous collidine or lutidine. The third group of elements could also be conveniently separated in *n*-butanol solvent mixture, Zn and Mo presenting marked contrast in appearance, when examined under ultra-violet light. While Boron occupied the outermost position in *n*-butanol solvent mixture and aqueous lutidine, it occupied the middle position in aqueous collidine. Ferron and sodium diethyl dithio carbamate were also quite useful as confirmatory spray reagents.

It would be of interest to mention that in the three mobile phases employed in these studies there was no significant deviation in the $R_f$ values of the cations, whether present individually, in mixtures or incorporated in Richard's medium diluted 1 : 2. Neat Richard's medium caused a slight diffusion and fading of the bands. Small fluctuations were, however, observed in certain cases and these could largely be attributed to the decrease in the rate of irrigation brought about by high cationic concentrations, which disappeared when the irriagtion was set up at the optimum level indicated earlier. In the concentrations employed the anions had no appreciable effect on the movement of the cations in the solvents used. A mixture of $CuCl_2.2H_2O$, $CuSO_4.5H_2O$, $Cu(CH_3COO)_2.H_2O$ and $Cu(NO_3)_2.3H_2O$ gave a single band of $R_f$ value 0·611 in *n*-butanol solvent mixture; besides, the individual salts also gave similar $R_f$ values. These

findings are not in agreement with those of Lewis and Griffiths (1951) who reported a marked influence of the anions on the movement of the cations. The need to control and maintain the rate of irrigation at an optimum level for sharp demarcation of the bands and also the reproducibility of the $R_f$ values has been emphasized earlier (Lakshminarayanan, 1954 *a*, *b*). It may also be pointed out that the H-ion concentration of the salt solutions spotted should be maintained at about 6·5 to 7·0 and precipitation of the cations avoided.

Work on the quantitative aspect of the present technique is under way and will be reported in due course.

## Acknowledgments

It is a pleasure to record my indebtedness to Professor T. S. Sadasivan for guidance and encouragement in the course of the work. My thanks are also due to my colleagues Mr. M. Lakshmanan and Mr. D. Subramanian for going through the manuscript and to the Government of India for the award of a senior research scholarship.

## Summary

A simple method for the chromatographic separation of inorganic cations in mixtures and in biological media in the order of 0·15 to 0·2 $\mu$g. has been described. Different spray reagents for the detection of the cations and also their $R_f$ values in three solvent mixtures, N-butanol-acetic acid-water, aqueous collidine and aqueous lutidine have been reported.

## References

Airan, J. W. .. "Application of circular chromatography to inorganic analysis," *Sci. Cult.*, 1952, **18**, 89.

——— and Barnabas, J. .. "Chromatographic separation of copper, cobalt and nickel," *Ibid.*, 1953, **18**, 438.

Giri, K. V. and Rao, N. A. N. "Circular paper chromatography. I. A technique for the separation and identification of amino acids, *J. Indian Inst. Sci.*, 1952*a*, **34**, 95.

——— .. "A technique for the identification of amino acids separated by circular paper chromatography, *Nature*, Lond., 1952 *b*, **169**, 923.

Lacourt, A., Sommereyns, G., Degeyndt, E., Baruh, J. and Gillard, J. "Quantitative inorganic paper chromatography. Sub-micro separation and determination of aluminium, iron and titanium" *Ibid.*, Lond., 1949, **163**, 999.

Lakshminarayanan, K. .. "A simple technique in paper disk chromatography," *Arch. Biochem. and Biophys.*, 1954*a*, **49** (2), 396.

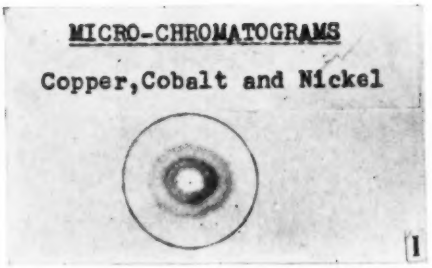
——— .. "Microchromatography. I. A technique for separation and identification of traces of amino acids, sugars etc.," *Ibid.*, 1954 *b*, **51** (2), 367.

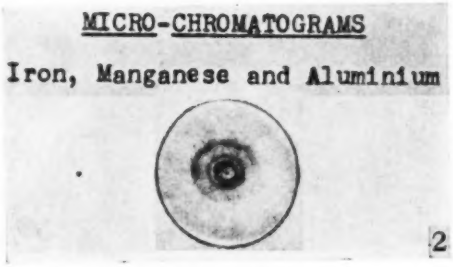
Lewis, J. A. and Griffiths, J. M. "Inorganic chromatography on cellulose. Part IV. Determination of inorganic compounds by paper strip separation and polarography," *Analyst*, 1951, **76**, 388.

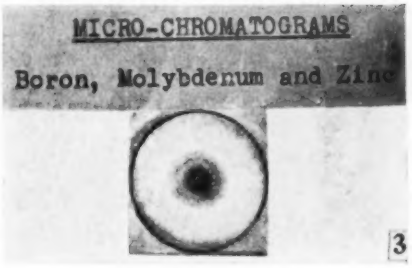
Pollard, F. H. and McOmie, J. F. W. "The analysis of inorganic compounds by paper chromatography," *Endeavour*, 1951, **10** (40), 213.

## PLATE IV

FIGS. 1–3. Microchromatograms showing the separation of cations of the three groups. (1) Cu, Co and Ni; (2) Fe, Mn and Al; (3) B, Mo and Zn. The bands represent the constituents in the order from periphery to centre. 0·15 to 0·2 $\mu$g. of each of the cations in 0·25 $\mu$l. spotted. Solvent mixture: N-butanol–acetic acid–water 4 : 1 : 5. Spray reagents: Rubeanic acid for Group (I) and 8-Hydroxyquinoline for groups II and III. Diameter of the solvent boundary: 2·3 cm. Time of irrigation: 45 min. Fig. 1. Photographed in ordinary light and Figs. 2 & 3 in ultra-violet light 3560 Å.


MICRO-CHROMATOGRAMS
Copper, Cobalt and Nickel
1


MICRO-CHROMATOGRAMS
Iron, Manganese and Aluminium
2


MICRO-CHROMATOGRAMS
Boron, Molybdenum and Zinc
3

FIGS. 1–3

# PHOTOPERIODISM IN RICE

## III. Effect of Short Day-Length on Four Late-Winter Varieties of Rice of Orissa

By Gadadhar Misra, M.Sc., D.Phil.
(*Department of Botany, Ravenshaw College, Cuttack-3*)

Received June 30, 1954
(Communicated by Dr. I. Banerji, D.Sc., F.A.Sc.)

### Introduction

The photoperiodic behaviour of different varieties of rice grown in various States of India has been studied by several workers. Saran (1945) investigated the photoperiodic response of five varieties of Bihar rice. He concluded that by application of short days any variety of rice, irrespective of class or maturity period to which it belonged, could be induced to flower within 60 to 63 days of germination. In Bengal, Sircar (1946), Sircar and Parija (1949) obtained great acceleration in flowering in three varieties of winter rice. In Uttar Pradesh, Misra (1950) obtained earlier flowering in two varieties of late rice by application of short days at different ages of the seedlings. The present investigation aimed at finding out the photoperiodic response of four late-winter varieties of rice of Orissa.

### Materials and Methods

Pure strains of the following varieties were kindly supplied by the Director, Central Rice Research Institute, Cuttack.

1. C. No. 3 (a selection from Kajal Champa of Cuttack District).
2. S.R. 26 B (a selection from Kalambank of Puri District. This is a salt-water resistant paddy).

3 and 4. F.R. 13 A and F.R. 43 B (Both are progenies of single straggling plants picked up from a field where rest of the plants had perished after heavy floods in 1933 in Orissa. These two are flood-resistant varieties).

A 10-hour short-day photoperiod was given to one week old seedlings for 3, 4, 5, and 6 weeks in the seed bed in separate experimental sets. After exposure to short-day photoperiods for these different durations the seedlings were transplanted along with the controls. In one set which received previously the short-day photoperiod for 6 weeks in the seed bed, the same treatment was continued till the first panicle emergence.

## EXPERIMENTAL RESULTS

*Time of Ear Emergence.*—The data on the period of panicle emergence are presented in Table I.

TABLE I

*Time from Sowing to First Panicle Emergence*

| | Days from sowing to ear emergence of main shoot | | | |
|---|---|---|---|---|
| | C. No. 3 | S.R. 26 B | F.R. 13 A | F.R. 43 B |
| Control .. .. | 142·20 | 136·30 | 137·45 | 137·90 |
| S. Day for 3 weeks .. | 145·20 | 138·50 | 137·80 | 141·80 |
| S. Day for 4 weeks .. | 148·70 | 142·70 | 138·35 | 142·40 |
| S. Day for 5 weeks .. | 149·20 | 144·15 | 140·10 | 142·70 |
| S. Day for 6 Weeks .. | 149·80 | 146·40 | 141·50 | 143·60 |
| S. Day prolonged till heading | 110·40 | 125·45 | 116·25 | 102·80 in 85% 69·60 in 15% |

Seed sown: June 18, 1949. Short-day treatment began: June 25, 1949. Seedlings transplanted: August 7, 1949.

Out of these four varieties, C. No. 3, S.R. 26 B and F.R. 13 A react to short-day photoperiod likewise and the other variety F.R. 43 B behaves differently. Short days for 3 weeks during the seed-bed stage bring about a delaying effect in ear emergence in the above stated three varieties. As the duration of the treatment increases to 4, 5, and 6 weeks there is more and more delay in the ear emergence. The interesting point is that when the short days have been given to the seedlings for 6 weeks and then continued after transplantation till ear emergence, a reverse effect, namely, a marked earliness in ear emergence is noted. Variety F.R. 43 B has behaved a little differently in that in the prolonged short-day treatment set, 85% of the plants have shown an average earliness of 35 days in ear emergence, while the rest 15% have shown an earliness of 68 days in ear emergence.

After about 4 months when photographs of the representative plants of F.R. 43 B were taken they revealed that the plants which were receiving 10-hr. short days till the time of ear emergence although flowered much earlier were very poor in vegetative growth whereas those in the control set which were receiving the full daylight of a natural day were in luxuriant vegetative condition and were at a stage when the ears are about to be emerged out of the boot.

*Tillers, Leaves and the Plant Height.*—Although records of number of tillers, number of leaves and the height of the plants were taken at regular intervals for four times during the life of the plants, only the records at the final stage are being presented in Table II, as the trend with regard to all these points was almost the same at all stages during the short-day treatments for different durations.

TABLE II

*Certain Morphological Observations following Short-Day Exposure of Seedlings for Different Periods of Four Paddy Varieties*

| | Short-day exposures for | | | | | |
|---|---|---|---|---|---|---|
| | Control | 3 weeks | 4 weeks | 5 weeks | 6 weeks | Prolonged till heading |
| | No. of tillers per plant | | | | | |
| C. No. 3 .. | 3·95 | 3·85 | 3·75 | 4·00 | 4·20 | 2·10 |
| S.R. 26 B .. | 3·55 | 3·60 | 3·65 | 3·40 | 3·50 | 2·05 |
| F.R. 13 A .. | 4·55 | 4·75 | 4·65 | 4·70 | 4·95 | 1·85 |
| F.R. 43 B .. | 4·90 | 5·70 | 5·40 | 5·10 | 6·50 | 2·40 |
| Mean .. .. | 4·23 | 4·47 | 4·36 | 4·30 | 4·78 | 2·10 |
| | No. of leaves per plant | | | | | |
| C. No. 3 .. | 13·70 | 12·00 | 11·15 | 15·49 | 16·25 | 8·70 |
| S.R. 26 B .. | 14·15 | 13·60 | 14·00 | 12·95 | 13·35 | 9·20 |
| F.R. 13 A .. | 18·10 | 20·45 | 18·70 | 21·20 | 23·05 | 11·05 |
| F.R. 43 B .. | 23·15 | 24·30 | 21·85 | 22·50 | 31·00 | 10·95 |
| Mean .. .. | 17·27 | 17·58 | 16·42 | 18·01 | 20·91 | 10·08 |
| | Height per plant in cm. | | | | | |
| C. No. 3 .. | 116·15 | 117·20 | 123·50 | 114·00 | 111·35 | 89·00 |
| S.R. 26 B .. | 125·70 | 120·85 | 130·10 | 121·25 | 115·45 | 86·00 |
| F.R. 13 A .. | 131·25 | 128·00 | 134·60 | 125·45 | 121·50 | 81·15 |
| F.R. 43 B .. | 125·85 | 117·10 | 116·80 | 116·05 | 115·35 | 83·00 |
| Mean .. | 124·73 | 120·78 | 126·25 | 119·18 | 115·91 | 84·78 |

A study of Table II clearly shows that—

(*a*) The tiller production on the whole has been a little better as a result of short-day treatments for 3, 4, 5, and 6 weeks in the seed bed. Tillering has been greatly reduced in all the varieties when the same treatment was prolonged till the time of heading.

(*b*) The production of leaves has not been appreciably changed from the controls by short-day treatments for 3, 4, 5, and 6 weeks in the seed bed. The production of leaves has been greatly suppressed by the short-day treatment when prolonged till ear emergence.

(*c*) The effect of the short-day treatment on height is not consistent with the duration of the treatment. The general trend is that height has not been materially affected by the treatments in the seed bed for various durations. The short-day treatment prolonged till ear emergence has considerably lowered the height of the plants in all the varieties.

*Grain Yield.*—The grains of the individual plants under the different treatments were collected separately and after the usual methods of drying were weighed. The data are graphically represented in Fig. 1. The data

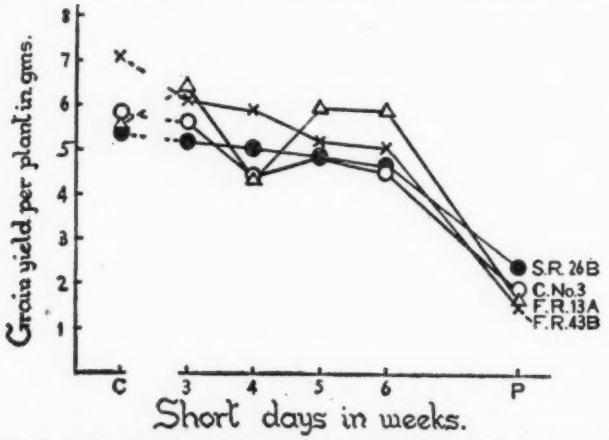



FIG. 1. Grain yield per plant in gm. of the four late-winter varieties C. No. 3, S.R. 26 B, F.R. 13 A and F.R. 43 B when the 7-day old seedlings were subjected to 10-hour short days for 3, 4, 5, 6 weeks and till ear emergence. The grain yield in prolonged treatment set (P) is considerably less in comparison with the control set (C) receiving full length of day.

on grain yield were also statistically analysed. The overall effect of the short-day treatments in the seed bed for 4, 5, and 6 weeks on all the varieties have reduced the yield of grains in comparison with the controls. The reduction is significant at 5% level. There is no significant difference in grain yield between the set receiving the treatment for 3 weeks at seed bed and the controls. A great reduction in grain yield is noted in the set receiving the same treatment till the time of ear emergence.

*Components of Yield.*—The components of yield such as number of panicles, length of panicle, number of spikelets per panicle, number of grains per panicle and the weight of one thousand grains are presented in Table III and the statistical analyses of the data in Table IV.

TABLE III

*Effect of Short-day Exposure on Panicle and Grain Characters*

| Rice variety | Control | Short-day exposures for 3 weeks | 4 weeks | 5 weeks | 6 weeks | Till heading |
|---|---|---|---|---|---|---|
| | | No. of panicles per plant | | | | |
| C. No. 3 .. | 3·30 | 3·15 | 3·10 | 3·40 | 3·50 | 2·05 |
| S.R. 26 B .. | 2·70 | 2·55 | 2·45 | 2·35 | 2·15 | 1·85 |
| F.R. 13 A .. | 3·80 | 3·70 | 2·95 | 3·65 | 4·00 | 1·75 |
| F.R. 43 B .. | 2·95 | 3·00 | 2·90 | 2·75 | 3·60 | 2·30 |
| Mean .. | 3·18 | 3·10 (−2·5) | 2·85 (−10·4) | 3·03 (−4·7) | 3·31 (+4·1) | 1·98 (−37·7) |
| | | Length of the panicle in cm. | | | | |
| C. No. 3 .. | 20·28 | 21·22 | 19·14 | 18·38 | 17·56 | 12·98 |
| S.R. 26 B .. | 20·30 | 18·78 | 19·72 | 19·80 | 19·72 | 14·90 |
| F.R. 13 A .. | 16·46 | 18·54 | 16·18 | 17·04 | 16·58 | 11·00 |
| F.R. 43 B .. | 18·92 | 20·36 | 19·22 | 19·52 | 19·16 | 12·14 |
| Mean .. | 18·99 | 19·72 (+3·8) | 18·56 (−2·3) | 18·68 (−1·7) | 18·25 (−3·9) | 12·75 (−32·9) |
| | | No. of grains per panicle | | | | |
| C.No. 3 .. | 87·12 | 88·46 | 88·64 | 78·10 | 73·88 | 57·36 |
| S.R. 26 B .. | 80·02 | 77·88 | 62·46 | 75·98 | 76·76 | 56·28 |
| F.R. 13 A .. | 64·64 | 76·80 | 65·78 | 75·26 | 69·46 | 49·94 |
| F.R. 43 B .. | 104·42 | 90·90 | 95·30 | 83·82 | 65·42 | 42·66 |
| Mean .. | 84·05 | 83·51 (−0·7) | 78·04 (−7·2) | 78·29 (−6·9) | 71·38 (−15·1) | 51·56 (−38·7) |
| | | No. of spikelets per panicle | | | | |
| C. No. 3 .. | 129·90 | 136·76 | 144·34 | 132·88 | 130·04 | 81·68 |
| S.R. 26 B .. | 129·50 | 135·02 | 129·78 | 146·28 | 138·70 | 100·24 |
| F.R. 13 A .. | 88·76 | 101·16 | 90·20 | 103·18 | 94·28 | 63·58 |
| F.R. 43 B .. | 127·64 | 144·36 | 149·82 | 136·62 | 123·78 | 69·82 |
| Mean .. | 118·95 | 129·32 (+8·7) | 128·53 (+8·0) | 129·74 (+9·1) | 121·70 (+2·3) | 78·83 (−33·7) |

TABLE III—(*Contd.*)

| Rice variety | Control | Short-day exposures for 3 weeks | 4 weeks | 5 weeks | 6 weeks | Till heading |
|---|---|---|---|---|---|---|
| | | *Percentage of Grains set per panicle* | | | | |
| C. No. 3 .. | 66·96 | 64·92 | 61·80 | 58·90 | 56·84 | 70·34 |
| S.R. 26 B .. | 61·66 | 57·54 | 49·20 | 50·98 | 55·52 | 56·10 |
| F.R. 13 A .. | 72·84 | 75·86 | 72·74 | 72·88 | 73·68 | 78·64 |
| F.R. 43 B .. | 81·76 | 65·66 | 63·58 | 65·42 | 52·70 | 63·78 |
| Mean .. | 70·80 | 65·99 (−6·8) | 61·83 (−12·7) | 62·04 (−12·4) | 59·68 (−15·7) | 67·21 (−5·1) |
| | | *One Thousand Grain Weight in gm.* | | | | |
| C. No. 3 .. | 18·82 | 18·54 | 18·42 | 18·12 | 17·58 | 16·48 |
| S.R. 26 B .. | 28·06 | 28·74 | 29·30 | 28·42 | 28·24 | 23·76 |
| F.R. 13 A .. | 22·96 | 22·50 | 22·64 | 21·72 | 21·28 | 19·38 |
| F.R. 43 B .. | 22·94 | 22·86 | 21·30 | 22·50 | 21·82 | 18·00 |
| Mean .. | 23·19 | 23·16 (−0·1) | 22·91 (−1·2) | 22·69 (−2·2) | 22·23 (−4·1) | 19·40 (−15·4) |

Figures in brackets indicate percentage increase (+) or decrease (−) from controls.

From a study of Tables III and IV it is clear that the prolonged short-day treatment has significantly reduced the number and length of panicles per plant but the other treatments have no significantly different effects from the controls. There is no difference in the number of grains per panicle between the control plants and those which received the short-day treatments for 3, 4, and 5 weeks in the seed bed. The number of grains in plants that received short-day treatment for 6 weeks and that in which the short days were prolonged till ear emergence are considerably reduced. The reduction is significant at 5 per cent. level. Unlike the numbers of grains, the number of total spikelets per panicle is more in the 3 week and the 5 week treated sets than the controls. There is no difference of the 4 week and 6 week sets from the controls. The prolonged short-day treatment, on the other hand, has reduced the total number of spikelets to a great extent. The percentage of grains set in per panicle is greatly reduced in the 3, 4, 5, and 6 week seed-bed treatment sets in comparison with the controls. In the prolonged short-day treatment set, however, the percentage of grain set in per panicle is maintained at a high level. The short-day treatments for 3, 4, 5, and 6 weeks

TABLE IV

*Analysis of Variance of the Data of Components of Yield*

| | D.F. | S.S. | M.S. | F. | 5% F. | 1%F. |
|---|---|---|---|---|---|---|
| | | No. of Panicles per Plant | | | | |
| Varieties (V) .. | 3 | 15·35 | 4·70 | 23·50** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 22·92 | 4·58 | 22·90** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 8·51 | 0·56 | 2·80** | 1·77 | 2·25 |
| Error .. | 96 | 19·55 | 0·20 | | | |
| TOTAL .. | 119 | 66·33 | | | | |
| | | Length of Panicle | | | | |
| Varieties (V) .. | 3 | 146·72 | 48·90 | 32·81** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 642·88 | 128·57 | 86·28** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 61·31 | 4·08 | 2·73** | 1·77 | 2·25 |
| Error .. | 96 | 143·78 | 1·49 | | | |
| TOTAL .. | 119 | 994·69 | | | | |
| | | No. of Grains per Panicle | | | | |
| Varieties (V) .. | 3 | 3594·39 | 1198·13 | 10·86** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 14705·74 | 2941·14 | 26·67** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 6574·55 | 438·30 | 3·97** | 1·77 | 2·25 |
| Error .. | 96 | 10584·22 | 110·25 | | | |
| TOTAL .. | 119 | 35458·90 | | | | |
| | | No. of Spikelets per Panicle | | | | |
| Varieties (V) .. | 3 | 30960·49 | 10320·16 | 40·74** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 38516·19 | 7703·23 | 30·41** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 6191·26 | 412·75 | 1·62 | 1·77 | 2·25 |
| Error .. | 96 | 24317·30 | 253·30 | | | |
| TOTAL .. | 119 | 99985·24 | | | | |
| | | Percentage of Grains Set per Panicle | | | | |
| Varieties (V) .. | 3 | 5649·03 | 1883·01 | 53·69** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 1712·88 | 342·57 | 9·76** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 1756·44 | 117·09 | 3·33** | 1·77 | 2·25 |
| Error .. | 96 | 3367·48 | 35·07 | | | |
| TOTAL .. | 119 | 12485·83 | | | | |
| | | One Thousand Grain Weight | | | | |
| Varieties (V) .. | 3 | 1473·61 | 491·20 | 442·52** | 2·70 | 3·98 |
| Treatments (T) .. | 5 | 208·99 | 41·79 | 37·64** | 2·30 | 3·21 |
| Interaction (V×T) | 15 | 39·38 | 2·62 | 2·36** | 1·77 | 2·25 |
| Error .. | 96 | 107·29 | 1·11 | | | |
| TOTAL .. | 119 | 1829·27 | | | | |

** Significant at 1% level.

duration at the seed-bed stage do not produce any appreciable change in the weight of the 1,000 grains but the prolonged short-day treatment has significantly lowered the value of the one thousand grain weight. We find that the decreased yield with prolonged treatment is brought about by decrease in all the yield components, size, number of panicles, number of grains per panicle and one thousand grain weight but the setting was not affected.

## DISCUSSION

The photoperiodic responses of the four late-winter varieties of rice, while exhibiting varied tendencies amongst themselves bring about certain interesting points in the developmental physiology of the rice plant. In all the four varieties there was a gradual delay in ear emergence under the short-day treatment from 3 to 6 weeks in the seed bed. In the treatment prolonged till ear emergence, however, there was a marked significant earliness.

Sircar and Parija (1949) by giving short days of 10 hours for 4, 5, and 6 weeks in the seed bed to ' Rupsail ', a winter variety of Bengal obtained ear emergence in the main shoot in 49, 47, and 50 days respectively as against 133 days in the controls. In ' Patnai ', another winter variety the time to ear emergence of the main shoot was 136, 79, and 80 days in 4, 5 and 6 weeks seed-bed treatment as against 136 days in the controls. In yet another winter variety ' Bhasamanik ', Sircar (1946) obtained flowering in 152·3, 154·7 and 152·5 days in the 4, 5, and 6 weeks seed-bed treatment as against 165 days in the controls. Of these three varieties studied by Sircar *et al.*, it is clear that ' Rupsail ' variety is a highly sensitive variety and ' Patnai ' a little less and ' Bhasamanik ' a much less sensitive variety. None of the four varieties studied in the present investigation is so supersensitive as the ' Rupsail ' variety. None of the four varieties have shown any photoperiodic induction in the way of earlier ear emergence, rather they have shown a delaying effect under the short-day treatments upto 6 weeks in the seed bed. The fact that prolonged treatment given to transplanted seedlings in continuation to the treatment they received in the seed bed clearly indicates that a stage comes in the ontogeny of the plant where a reverse change from delaying effect to earliness takes place. It is, therefore, very clear that the effect of short days in effecting earlier ear emergence is by no means of a quantitative nature. This is in contrast with the results obtained by Sircar (1946) with ' Bhasamanik ' and Sircar and Sen (1953) with ' Rupsail '.

The differential behaviour between treatments in the seed bed upto 6 weeks and that continued after transplantation beyond the 6th week stage is possibly due to the fact that short days are only effective in bringing about earliness when applied at or beyond a particular stage of the plant. Without

further work it is not possible to say precisely at what particular stage the change-over from delaying to earliness results. It is also to be considered whether the temperature prevailing upto 6 weeks from the day of treatment initiation and after seed-bed stage is responsible for this change.

It would be interesting to continue the short-day treatments for 7, 8, 9 weeks and so on till the real change where the transition from delay to earliness takes place is clearly noted. Exactly a similar phenomenon though of opposite nature was recorded by Purvis and Gregory (1937). With the exposure of seedlings of winter rye to preliminary short days of 10 hours per day the magnitude of the effect on flowering increased with length of treatment upto 6 weeks, but longer exposures retarded flowering.

Although prolonged short days brought about a highly significant earlier heading it is important to note that the average number of tillers, leaves, and plant height have been very much reduced and the low level is maintained throughout the life period of the plant. Similar effects in retardation of growth as a result of the application of the photoperiods is of quite common occurrence (Austin, 1935; Garner and Allard, 1923; Murneek, 1936, 1937; Quinby and Karper, 1947). Quinby and Karper (1947) working with 19 sorghum varieties and 21 first generation hybrids noted in every case that tillering was greatly reduced by short-photoperiod treatment. As short-photoperiod treatment was discontinued after floral initiation, many plants of tillering varieties tillered even at that late stage. Such tillers were not affected by the fact that the main plant had been florally inducted but grew into large tillers similar to those that develop normally. Exactly such behaviour in the suppression of tillering was noted in all these varieties of rice under observation.

## Summary

The influence of 10 hour short-day photoperiod (8–00 A.M. to 6–00 P.M.) has been studied on four late-winter varieties of rice, C. No. 3 (a selection from Kajal Champa of Cuttack District), S.R. 26 B (a selection from Kalambank of Puri District), F.R. 13 A and F.R. 43 B (both flood-resistant varieties) of Orissa. Out of these four varieties, C. No. 3, S.R. 26 B and F.R. 13 A react to short-day photoperiod likewise and the other variety F.R. 43 B behaves differently. Short days for 3 weeks during the seed-bed stage bring about a delaying effect in ear emergence in the above stated three varieties. As the duration of short-day treatment increases to 4, 5, and 6 weeks in the seed bed, there is more and more delay in the time of ear emergence. The interesting point is that when the short-day photoperiod has been given to the seedlings for 6 weeks and then continued after transplantation

till ear emergence, a reverse effect, namely, a marked earliness in ear emergence is noted. Variety F.R. 43 B has behaved a little differently. In the prolonged short-day treatment set, 85% of the plants have shown an average earliness of 35 days in ear emergence, while the rest 15% have shown an earliness of 68 days in ear emergence.

ACKNOWLEDGEMENT

I express my indebtedness to Prof. Shri Ranjan for his guidance and stimulating criticism throughout the progress of the work at the Botanical Laboratory of the Allahabad University. I am thankful to Mr. T. P. Abraham, former Statistician, Central Rice Research Institute, Cuttack, for his advice with regard to the statistical analysis of the experimental data and to Mr. C. M. Bastia for his help in the preparation of the diagram. My thanks are due to Prof. B. Samantarai, Ravenshaw College, Cuttack, for kindly going through the manuscript and offering suggestions for its improvement.

LITERATURE CITED

Austin, J. P. .. "Some effects of the photoperiod on development of *Impatiens balsamea*," *Pl. Phys.*, 1935, **10**, 545–52.

Garner, W. W. and Allard, H. A. "Further studies in photoperiodism. The response of the plant to relative length of day and night," *Jour. Agr. Res.*, 1923, **23**, 871–920.

Misra, G. .. "Effect of photoperiod on the flowering time of two late varieties of paddy," *Curr. Sci.*, 1950, **19**, 126–27.

Murneek, A. E. .. "A separation of certain types of response of plants to photoperiod," *Proc. Amer. Soc. Hort. Sci.*, 1936, **34**, 507–09.

——— .. "Biochemical studies of photoperiodism in plants," *Missouri Agr. Exp. Sta. Res. Bull.*, 1937, **No. 268**, 84 pp.

Purvis, O. N. and Gregory, F. G. "Studies in vernalisation of cereals, I. A comparative study of vernalisation of winter rye by low temperature and by short days," *Ann. Bot.* (N.S.), 1937, **1**, 569–91.

Quinby, J. R. and Karper, R. E. .. "The effect of short photoperiod on sorghum varieties and first generation hybrids," *Jour. Agr. Res.*, 1947, **75**, 295–300.

Saran, A. B. .. "Studies on the effect of 'short' and 'long day' treatment on the growth period and the flowering dates of different paddy varieties," *Jour. Ind. Bot. Soc.*, 1945, **24**, 153–61.

Sircar, S. M. .. "Studies in the Physiology of rice, III. Vernalisation by short days," *Proc. Nat. Inst. Sci.* (India), 1946, **12**, 191–98.

—— and Parija, B. .. "Studies in the physiology of rice, V. Vernalisation and photoperiodic response in five varieties," *ibid.*, 1949, **15**, 93–107.

—— and Sen, S. P. .. "Studies on the physiology of rice, VI. Effect of photoperiod on development of the shoot apex," *Bot. Gaz.*, 1953, **114**, 436–48.

# STUDIES ON INDIAN ICHNEUMONIDAE (HYMENOPTERA)

## Subfamily BANCHINAE. Part II: tribe LISSONOTINI

By E. S. Narayanan, M.A., Ph.D. (Lond.), D.I.C., F.R.E.S., F.E.S.I., F.A.Sc.

and

Kundan Lal, M.Sc. (Hons.)

*(Indian Agricultural Research Institute, New Delhi)*

Received April 29, 1954

Contents

| | Page |
|---|---|
| The Tribe Lissonotini .. .. .. .. | 183 |
| The Genus *Stictolissonota* Cameron .. .. .. | 184 |
| The Genus *Amersibia* Förster .. .. .. | 184 |
| The Genus *Miophatnus* Cameron .. .. .. | 185 |
| The Genus *Lissonota* Gravenhorst .. .. .. | 185 |
| The Genus *Ctenopimpla* Cameron .. .. .. | 186 |
| The Genus *Meyva* Cameron .. .. .. | 187 |
| The Genus *Syzeuctus* Fœrster .. .. .. | 187 |
| The Genus *Lampronota* Curtis .. .. .. | 189 |

## SYSTEMATICALLY ARRANGED CATALOGUE OF INDIAN SPECIES

### Tribe 2. LISSONOTINI

Members of this tribe are parasites of caterpillars in burrows, leaf rolls and other retreats.

*Lissonotini* Ashmead, *Proc. ent. Soc., Wash.*, **3**: 278, (1893–96). Schmiedeknecht, *Genera Insect., fas.* **62**: 70, (1907). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 442, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 310, (1951).

*Lissonotides* Morley, *Ichn. Brit.*, **3**: 171, (1907); *Fauna Brit. India, Hymen.*, **3**: 215, (1913).

**Stictolissonota** Cameron

*Stictolissonota* Cameron, *Tijdschr. Ent.*, **50**: 106, (1907), (Type of the genus: *S. foveata* Cam.). Morley, *Fauna Brit. India, Hymen.*, **3**: 216, (1913).[1] [1]Sikkim.

**foveata** Cameron

*Stictolissonota foveata* Cameron, *Tijdschr. Ent.*, **50**: 107, (1907). ♀. Morley, *Fauna Brit. Indian, Hymen.*, **3**: 217, (1913). ♀.[1] [1]Sikkim.

**bicolor** Rao

*Stictolissonota bicolor* Rao, *Indian For. Rec., Ent. Ser.*, **8** (8): 182, (1953). ♀.[1] [1]U.P.: Dehra Dun.

**Amersibia** Förster

*Amersibia* Förster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 167, (1868), (Type of the genus: *Meniscus* (*Amersibia*) *variipes* Szepligeti). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 442, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 310, (1951).

*Alloplasta* Fœrster, *Verh. Naturh. Ver. Rheinl.*, **25:** 167, (1868). Morley, *Fauna Brit. India, Hymen.*, **3:** 222, (1913).[1] [1]Range. Europe, Punjab: Simla.

Trichopimpla Cameron, *Zeits. Hymen-Dip.*, **3**: 303, (1903).

**pilosa** (Cameron)

*Trichopimpla pilosa* Cameron, *Zeits. Hymen-Dip.*, **3**: 303, (1903). ♀.

*Alloplasta pilosa* Morley, *Fauna Brit. India, Hymen.*, **3**: 223, (1913). ♀.[1] [1]Punjab: Simla.

*Amersibia* (= *Alloplasta*) *pilosa* Townes, *Mem. Amer. ent. Soc.*, **11** (1): 442, (1944).

**Miophatnus** Cameron

*Miophatnus* Cameron, *Tijdschr. Ent.*, **50**: 105, (1907), (Type of the genus: *M. nigromaculatus* Cameron). Morley, *Fauna Brit. India, Hymen.*, **3**: 204, (1913).[1] [1]Sikkim.

**nigromaculatus** Cameron

*Miophatnus nigromaculatus* Cameron, *Tijdschr. Ent.*, **50**: 105, (1907). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 205, (1913).[1] [1]Sikkim.

**Lissonota** Gravenhorst

*Lissonota* Gravenhorst, *Ichn. Eur.*, **3**: 30, (1829), (Type of the genus: *L. sulphurifera* Grav.). Dalla Torre, *Cat. Hymen.*, **3**: 492, (1901–02). Schmiedeknecht, *Genera Insect., fas.* **62**: 89, (1907). Morley, *Ichn. Brit.*, **3**: 184, (1907); *Fauna Brit. India, Hymen.*, **3**: 224, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (1): 447, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 311, (1951). [1]Both hemispheres.

*Stilbonota* Stephens, *Ill. Brit. Ent.*, **7**: 126, (1835).

*Asynida* Gistel, *Naturg. Thierr.*, P. IX.

*Echthrodoca* Schmiedeknecht, *Zool. Jahrb.* Syst., **13**: 301, 303, 307, 326, 328, (1900).

**binghami** Cameron

*Lissonota binghami* Cameron, *Tijdschr. Ent.*, **50**: 109, (1907). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 229, (1913).[1] [1]Sikkim.

**brevicauda** Morley

*Lissonota brevicauda* Morley, *Fauna Brit. India, Hymen.*, **3**: 225, (1913). ♀.[1] [1]Sikkim; Nicobar Island.

**greeni** Cameron.

*Lissonota greeni* Cameron, *Spolia Zeylan.*, P. 142, (1905). ♀. Schmiedeknecht, *Genero Insect., fas.* **62**: 91, (1907). Morley, *Fauna Brit. India, Hymen.*, **3**: 230, (1913). ♀,[1] Ceylon: Peradeniya.

**lepida** Cameron

*Lissonote lepida* Cameron, *Zeits. Hymen-Dip.*, **8**: 43, (1908). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 227, (1913). ♂.[1] [1]Sikkim.

**minuenta** Morley

*Lissonota minuenta* Morley, *Fauna Brit. India, Hymen.*, **3**: 228, (1913). ♀.[1] [1]Sikkim: Darjeeling

**morum** Morley

*Lissonota morum* Morley, *Fauna Brit. India, Hymen.*, **3**: 230, (1913). ♀.[1] [1]Assam: Ukhrul in Manipure.

**spilopus** Cameron

*Lissonota spilopus* Cameron, *Zeit. Hymen-Dip.*, **8**: 42, (1908). ♂. Morley, *Fauna Brit. India, Hymen.*, **3**: 227, (1913). ♂.[1] [1]Sikkim.

**v-maculata** Cameron

*Lissonota v-maculata* Cameron, *Tijdschr. Ent.*, **50**: 108, (1907). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 229, (1913).[1] [1]Sikkim.

**Ctenopimpla** Cameron

*Ctenopimpla* Cameron, *Manch. Mem.*, p. 189, (1899), (Type of the genus: *C. albomaculata* Cam.). Dalla Torre, *Cat. Hymen.*, **3**: 489, (1901–02). Schmiedeknecht, *Genera Insect., fas.*, **62**: 88, (1907). Morley, *Fauna Brit. India, Hymen.*, **3**: 231, (1913).[1] [1]Range. Assam.

**albomaculata** Cameron

*Ctenopimpla albomaculata* Cameron, *Manch. Mem.*, p. 190, (1899). ♀. Morley, *Fauna Brit. India, Hymen.*, **3**: 231, (1913). ♀.[1] [1]Assam: Khasi Hills,

### Meyva* Cameron

*Meyva* Cameron, *Manch. Mem.*, p. 191, (1899), (Type of the genus: *M. villosa* Cam.). Dalla Torre, *Cat. Hymen.*, **3**: 487, (1901–02). Schmiedeknecht, *Genera Insect., fas.* **62**: 93, (1907). Morley, *Fauna Brit. India, Hymen.*, **3**: 232, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (1): 461, (1944).

[1]Range. Assam.

**villosa** Cameron

*Meyva villosa* Cameron, *Manch. Mem.*, p. 192, Pl. 3, Fig. 11 (wing), (1899). Dalla Torre, *Cat. Hymen.*, **3**: 487, (1901–02). Schmiedeknecht, *Genera Insect., fas.*, **62**: 93, (1907). Morley, *Fauna Brit. India, Hymen.*, **3**: 233, (1913). ♂.[1]

[1]Assam: Khasi Hills.

### Syzeuctus Fœrster

*Diceratops* Fœrster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 167, (1868).

*Syzeuctus* Fœrster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 167, (1868), (Type of the genus: *Ichneumon maculatorius* F.). Dalla Torre, *Cat. Hymen.*, **3**: 505, (1901–02). Schmiedeknecht, *Genera Insect., fas.* **62**: 82, (1907). Morley, *Fauna Brit. India, Hymen.*, **3**: 234, (1913).[1] Dutt, *Mem. Dep. Agric. India, Ent.*, **8**: (2): 16, (1923). Townes, *Mem. Amer. ent. Soc.*, **11** (1): 461, (1944). Muesebeck, Krombein, Townes and others, *U.S. Dep. Agric. Monograph*, **2**: 315, (1951).

[1]Range. Europe-Asia.

*Syzeucta* Thomson, *Opusc. ent.*, **13**: 1415, (1899).

*Ephialtina* Szepligeti, *Sjöstedt's Kilimandjaro-Meru Exped.*, **8**: 74, (1908).

**annulipes** (Cameron)

*Mesoleptus annulipes* Cameron, *Manch. Mem.*, p. 103, (1900). ♂.

---

* Townes in his *Mem. Amer. Ent. Soc.*, **11**, 1944, has treated *Meyva* Cameron as synonym of *Syzeuctus* Forst. with query mark. But we have carefully gone through the characters of both genera. In our opinion we do not consider the genus *Meyva* Cameron as synonym of *Syzeuctus* Forst. So in these studies we have put these two genera separately though near to each other.

*Syzeuctus annulipes*, Morley, *Fauna Brit. India, Hymen*, **3**: 236, (1913). ♀.[1] Dutt, *Mem. Dep. Agric. India. Ent.*, **8** (2): 16, (1923).[2]

[1]Assam: Khasi Hills; Bengal: Pusa, Chapra, Adra, Monghur, Karmatar, Bhogaon, and Calcutta; Madras: Mysore, Tenmalai, Travancore; Ceylon: Pundaluoya, Colombo, Peradeniya and Madulsima. Queensland.
[2]Bihar: Pusa, Chapra; Assam: Shillong.

**baluchistanensis** (Cameron)

*Lissonota baluchistanensis* Cameron, *J. Bombay nat. Hist. Soc.*, **17**: 284, (1901). ♀.

*Lissonota apicipennis* Cameron, *J. Bombay nat. Hist. Soc.*, **14**: 426, (1902). ♀.

*Syzeuctus baluchistanensis* Morley, *Fauna Brit. India, Hymen.*, **3**: 239, (1913). ♀.[1]

[1]Baluchistan: Quetta.

**claripennis** (Cameron)

*Lissonota claripennis* Cameron, *J. Bombay nat. Hist. Soc.*, **14**: 127, (1902). ♂.

*Syzeuctus claripennis* Morley, *Fauna. Brit. India, Hymen.*, **3**: 235, (1913). ♂.[1]

[1]Punjab: Simla; Madras: Madras; Ceylon: Heneratgoda.

**compressus** Morley

*Syzeuctus compressus* Morley, *Fauna Brit. India, Hymen.*, **3**: 234, (1913). ♀.[1] [1]Sikkim.

**Zanthorius** (Cameron)

*Lissonota zanthorius* Cameron, *J. Bombay nat. Hist. Soc.*, **14**: 426, (1902–03). ♂.

*Syzeuctus zanthorius* Morley, *Fauna Brit. India, Hymen.*, **3**: 238, (1913). ♂.[1]. Dutt, *Mem. Dep. Agric. India, Ent.*, **8** (2): 16, (1923).[2] [1]Punjab; Bengal: Pusa and Chapra; Central India; Bombay: Deesa, Poona and Bombay. [2]Bihar: Pusa, Chapra.

**Lampronota** Curtis

*Lampronota* Curtis, *Brit. Ent.*, **9**: 407, (1832), (Type of the genus: *Ichneumon setosus* Fourcroy). Haliday, *Ann. nat. Hist.*, **2**: 120, (1839). Morley, *Fauna Brit. India, Hymen.*, **3**: 240, (1913).[1] Townes, *Mem. Amer. ent. Soc.*, **11** (1): 444, (1944). [1]North America, Cuba, Europe, Sikkim.

*Meniscus* Schiödte, *Guérin's Mag. Zool. Ins.* **9**: 10, (1839).

*Bothynophrys* Fœrster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 166, (1868).

*Bathycetes* Fœrster, *Verh. Naturh. Ver. Preuss. Rheinl.*, **25**: 167, (1868).

*Stilbopoides* Rhower, *Proc. ent. Soc. Wash*, **15**: 183, (1913).

**quintana** Morley

*Lampronota quintana* Morley, *Fauna Brit. India, Hymen.*, **3**: 241 (1913). ♂.[1] [1]Sikkim.

ACKNOWLEDGEMENT

Taxonomic work of this important parasitic family Ichneumonidae has been made possible under a generous grant sanctioned by the Indian Council of Agricultural Research. We wish to thank Dr. B. P. Pal, Director, Indian Agricultural Research Institute, for the keen interest he has taken in this piece of research work. We are indebted to Prof. Townes for his valuable suggestions and comments. We are also grateful to Dr. O. W. Richards of the Imperial College of Science and Technology, London, for going through the manuscript and making valuable corrections.

1609–54 Printed at The Bangalore Press, Bangalore City, by C. Vasudeva Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.

# INDEX TO VOL. XL (B)

## AUTHOR INDEX


Agarwala, R. K. .. *See* Tandon and Agarwala.

Appanna, T. C. and Devadatta S. C. — The effect of prawn protein on the bone of mature rats, 44.

Devadatta, S. C. .. *See* Appanna and Devadatta.

Gokhale G. P., Kibe, M. M. and Narayana, N. — Soil amendments and phosphate availability, 116.

Jayaraman, R. .. *See* Prasad and Jayaraman.

Kibe, M. M. .. *See* Gokhale and others.

Lakshminarayanan, K. .. Microchromatography, II, 167.

Lal, Kundan .. *See* Narayanan and Lal.

Mallik, A. K., Subramanian, V. K. and Venkatraman, R. — Complete enumeration experiment in groundnut crop for developing the optimum shape and size of samples for crop weather observations, 8.

Misra, Gadadhar .. Photoperiodism in rice, III, 173.

Nagaraj, M. .. Desoxyribonucleic acid (DNA) content in the female gametophyte and nucellus of *Rhæo discolor*, 110.

Narayana, N. .. *See* Gokhale and others.

Narayanan, E. S. and Lal, Kundan — Studies on Indian Ichneumonidæ (hymenoptera) Sub-family: Banchinæ, I, II, 38, 183.

Nayar, K. K. .. The neurosecretory system of the fruit fly *Chætodacus cucurbitæ* Coq., I, 138.

Prasad, R. Raghu and Jayaraman, R. — Preliminary studies on certain changes in the plankton and hydrological conditions associated with the swarming of *noctiluca*, 49.

Raheja, P. C. and Sekhon, G. S. — Growth studies in *Saccharum officinarum*, VII, 25.

Raja Bai Naidu, K. G. .. The post-larval development of the shore crab *Ocypoda platytarsis* M. Edwards and *Ocypoda cordimana* Desmarest, 89.

Ramakrishnan, T. S. and Sundaram, N. V. — Additions to fungi of Madras, XVI, 17.

Saraswathi Devi, L. .. Bio-assay of heavy metals by *Aspergillus niger*—sensitivity of a new strain, 1.

Sekhon,G. S. .. *See* Raheja and Sekhon.

| | | |
|---|---|---|
| Singh, Inderjit | .. | *See* Singh and Singh. |
| Singh, K. S. | .. | *See* Thapar and Singh. |
| Singh, Sunita Inderjit and Singh, Inderjit | | The effect of ions on the contractile mechanism of unstriated muscle and its relation to tonus, 125. The intimate nature of muscular contraction, 145. |
| Sreenivasan, A. | .. | *See* Venkataraman and Sreenivasan. |
| Subramanian, V. K. | .. | *See* Mallik and others. |
| Sundaram, N. V. | .. | *See* Ramakrishnan and Sundaram. |
| Sundara Raj, B. | .. | The problem of the apparent discontinuous distribution of *Harpodon nehereus* (Hamilton), 58. |
| Tandon, R. N. and Agarwala, R. K. | | Pathological studies of *Glæosporium psidii* causing die-back of guavas, 102. |
| Thapar, G. S. and Singh, K. S. | | Studies on the life-history of *Trichuris ovis* (Abildgaard, 1795) (Fam. Trichuridæ: Nematoda), 69. |
| Venkatraman, R. | .. | *See* Mallik and others. |
| Venkataraman, R. and Sreenivasan, A. | | Bacteriology of off-shore sea-water of the West Coast, 161. |

## TITLE INDEX

*Chætodacus cucurbitæ* Coq., fruit fly, the neurosecretory system, I (Nayar), 138.

Desoxyribonucleic acid (DNA) content in the female gametophyte and nucellus of *Rhæo discolor* (Nagaraj), 110.

Fungi of Madras, additions, XVI (Ramakrishnan and Sundaram), 17.

*Glæosporium psidii* causing die-back of guavas, pathological studies (Tandon and Agarwala), 102.

Groundnut crop, complete enumeration experiment, for developing the optimum shape and size of samples for crop weather observations (Mallik and others), 8.

*Harpodon nehereus* (Hamilton), the problem of the apparent discontinuous distribution (Sundara Raj), 58.

Heavy metals, bio-assay, by *Aspergillus niger*—sensitivity of a new strain (Saraswathi Devi), 1.

Ichneumonidæ (hymenoptera), Indian, studies, Sub-family: Banchinæ, I, II (Narayanan and Lal), 38, 183.

Microchromatography, II (Lakshminarayanan), 167.

Muscle, unstriated, the effect of ions on the contractile mechanism, and its relation to tonus (Singh and Singh), 125.

Muscular contraction, the intimate nature (Singh and Singh), 145.

*Noctiluca*, swarming, certain changes in the plankton and hydrological conditions associated with, preliminary studies (Prasad and Jayaraman), 49.

*Ocypoda platytarsis* M. Edwards and *Ocypoda cordimana* Desmarest, shore crab, the post-larval development (Raja Bai Naidu), 89.

Photoperiodism in rice, III (Misra), 173.

Prawn protein, the effect, on the bone of mature rats (Appanna and Devadatta), 44.

*Saccharum officinarum*, growth studies, VII (Raheja and Sekhon), 25.

Sea-water, offshore, of the West Coast, bacteriology (Venkataraman and Sreenivasan), 161.

Soil amendments and phosphate availability (Gokhale and others), 116.

*Trichuris ovis* (Abildgaard, 1795) (Fam. Trichuridæ: Nematoda), studies on the life-history (Thapar and Singh), 69.